CONVECTIONLESS ELECTROPHORETIC SEPARATION OF BIOLOGICAL PREPARATIONS

by

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I. INTRODUCTION

Electrophoresis is a separation technique used for analysis of delicate and complex mixtures of biological materials, for purifying biochemical products, and for medical diagnosis. Two methods of electrophoresis are in use: the free boundary or moving boundary method carried out without the use of a stabilizing medium and the zone method that requires a stabilizing medium. In low molecular-weight materials the zone method is usually satisfactory but is impractical for high molecular weight materials because of immobility in the stabilizing media.

While electrophoresis is almost indispensible to the clinical analyst, it has not proven very useful as a means of producing pure biologicals in significant amounts, due largely to problems with convection and sedimentation (both due to gravity) in large-scale operations. It was suggested several years ago that free electrophoresis in orbiting space vehicles (where there is essentially zero gravity) may make possible the production of pure biologicals and vaccines. It may also be possible to achieve the clean separation of thymus-dependent, T, lymphocytes from thymus-independent, B, lymphocytes, or the clean separation of bone marrow stem from bone marrow graft-vs.-host reactive cells. Such separations could be of inestimable value to researchers who are looking for cures or vaccines for cancer or leukemia.

Attempts have been made to use free electrophoresis for separation of high molecular-weight materials which have little or no mobility on a support. In the presence of gravity, however, the method suffers primarily in stabilization of the boundaries of the migrating ions and in incomplete separations, largely due to density differences between the solvent and the solute. In addition, large-scale separations are limited by thermal convection arising from Joule heating of the solution.

This work was directed toward development of equipment, and definition of requirements, materials of construction, and operating conditions for a zero-g electrophoresis apparatus to demonstrate certain basic principles. The Apollo 16 demonstration apparatus was, of course, not a prototype of future preparative equipments which may be used for the purification of significant biological materials in space. Major areas of concern were the space, weight, and power requirements for an apparatus which, while simple, might be capable of providing information valuable to the development of future useful zero-g electrophoresis equipments.

Electrophoresis was demonstrated on Apollo 14 when red and blue dyes were separated on the return trip from the moon (Ref. 1). Photographs showed that the boundary dividing the dyes was sharper and better defined than on earth. The apparatus also contained samples of hemoglobin and DNA which were not observed to migrate. Subsequent examination of the apparatus indicated that these specimens were destroyed by bacteria, probably during the long storage time before the demonstration in space.

Ref. 1. E. C. McKannan, et al, "Electrophoresis Separation in Space-Apollo 14", NASA TM X-64611, August 29, 1971.

The Apollo 14 experiment demonstrated that most of the component parts of the apparatus worked as designed. The electrical and electrolyte circulation systems of the apparatus operated successfully and gas bubbles generated at the electrodes were filtered and absorbed as planned. Sample injection was accomplished, although a sliding valve did not fully open due to a mechanical misalignment. In summary, much was learned on Apollo 14 about the problems and requirements for doing electrophoresis in space.

The Apollo 14 electrophoresis unit consisted of a transparent polymeric block with several tubular holes for electrophoresis cells. Experimentation showed the need for recirculation of the electrolyte to remove bubbles resulting from electrolysis of the solvent, and to minimize changes in the pH of the electrode compartments. The electrolyte in the anode and cathode compartments was interchanged with a peristaltic or tubing pump. Effluent from the anode compartment was pumped to the cathode compartment and vice versa. Current leakage through this system was negligible.

The objective of the Apollo 16 experiment was the electrophoresis of large, dense particles in a liquid medium. Polystyrene microspheres of two different sizes were used as a model material to demonstrate that scientifically significant biological materials can be electrophoretically separated in space.

The apparatus was generally similar to that used on Apollo 14, but incorporated changes to improve sample visibility and photography, to provide more data on experiment parameters, and to provide increased reliability in operation. The purpose of this report is to describe the Apollo 16 Electrophoresis Demonstration equipment and experiment, and to detail the ground-based testing incident to its design.

II. GROUND-BASED TESTS AND DEVELOPMENTS

Efforts during the early part of the contract period were directed primarily at conducting tests in electrophoresis cells of the Apollo 14 type; at modifying the Apollo 14 equipment to improve its performance and reliability; at defining samples which may remain stable during storage; and at developing a protocol for the sterilization and aseptic loading of electrophoresis equipment for use in space.

A. Modification of Apollo 14 Apparatus

The cell or cell block used on the Apollo 14 electrophoresis apparatus had two principal deficiencies. Being mostly machined from a single block of Lexan for physical strength, and having bonded on the outside a piece of tempered glass to which was further bonded a layer of Teflon, the Apollo 14 block had rather marginal optical properties. Furthermore, the general design made it impossible to obtain side lighting as was desired on Apollo 16.

In the Apollo 14 apparatus, sample injection was accomplished by motion of a screw-driven slide. This required precise alignment of the activating screw and the end of the slide. It led to the possibility of disturbance of the sample due to the motion.

The sample injection or slide valve used in the Apollo 14 electrophoresis block was greatly improved by a simple modification. In the new design the injection slide remains stationary. The sample is retained in the injection slide by a dialysis membrane on one side and a removable Kapton film on the other. Sample "injection" is accomplished by pulling out the Kapton film. The only disturbance to the sample and to the cell electrolyte is the skin effect of the slowly moving Kapton strip. Furthermore, both the sample and the cell electrolyte are at all times retained by the "O" rings which surround the electrophoresis cell.

Other changes were also designed such as changes in the electrode and electrode compartment configurations, and the use of a tubular structure (as opposed to a drilled-out block) of the type used for the Apollo 16 electrophoresis demonstration.

Experiments conducted in Apollo 14 electrophoresis blocks led to modifications of the block for use on earth. Results, however, indicated that these modifications were not adequate. Another cell was therefore built which is more suited for ground operation but which incorporates all the important features of the Apollo 14 cell - size, material, sample injection (modified), buffer system, and voltage.

B. Electrophoresis Experiments

As was mentioned above, electrophoresis experiments led us to the conclusion that cells of the Apollo 14 type are not suitable for testing on earth. This may seem surprising in light of the number of experiments that were conducted in just such cells prior to the Apollo 14 demonstration. It must be remembered, though, that all the electrophoresis experiments preliminary to Apollo 14 were conducted with dissolved samples where the particles were molecular. The experiments in the present work were conducted with polystyrene latexes having particle sizes of 0.1 to 0.8 micrometers, and were much more difficult to perform in 1 g. All these experiments were conducted in sucrose density gradients, formed by either one of two machines. One operates on a principle developed by Bock and Ling (Reference 2) and independently by Lakshmanan and Lieberman (Reference 3), and is designed to produce a linear gradient. The other machine is of our own design. The exact nature of the gradient produced by this machine has not been measured, but in practice we have seen no significant different in the usefulness of the two types of gradient. Separations of 0.109μ and 0.357μ polystyrene latexes were attempted at pH 7.5, 8.5, and 9.0, and at buffer concentrations of 0.0015M and 0.003M. At least a partial separation of the two latexes was achieved

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2. Bock, R.M. and Ling, N.S., Anal. Chem. 26, 1543 (1954).

3. Lakshmanan, T.K. and Liebermann, S., Arch. Biochem. Biophys. 53, 258 (1954).

in 0.003M tris/HCl buffer at pH 8.5 with a linear 20% sucrose density gradient. The major problems that were encountered were due to convection in spite of the density gradient, matching the sample density with that of the buffer at the point of injection, and a peculiar phenomenon in which streamers of high density sample drift downward in the tube (See Figure 1). This is probably similar to the instability in some protein separations reported by Brakke (Reference 4) and is therefore of little concern, except as an experimental difficulty in the ground-based investigations.

Twenty-three electrophoresis experiments were conducted in a modified Apollo 14 equipment with polystyrene latexes as sample materials. Some of the expected experimental difficulties included the development of a density gradient which provided neutral buoyancy of the sample at injection without excessive buoyancy lower in the cell, and the occasional formation of an air bubble in the cell. The latter problem was solved by a change in cell design; the former by practice. Unexpected difficulties, however, also occurred. Primary among these were the formation of streamers and a lack of repeatability in ostensibly replicate runs. Photomicrographs of leading and trailing ends of a latex sample may have provided the explanation for these problems. The photomicrographs showed the presence of numerous agglomerates of latex particles, so in subsequent tests a small amount of wetting agent was added to the solution (See Figure 2). The addition of wetting agent improved

4. Brakke, M.K., Arch. Biochem. Biophys., 55, 175 (1955).

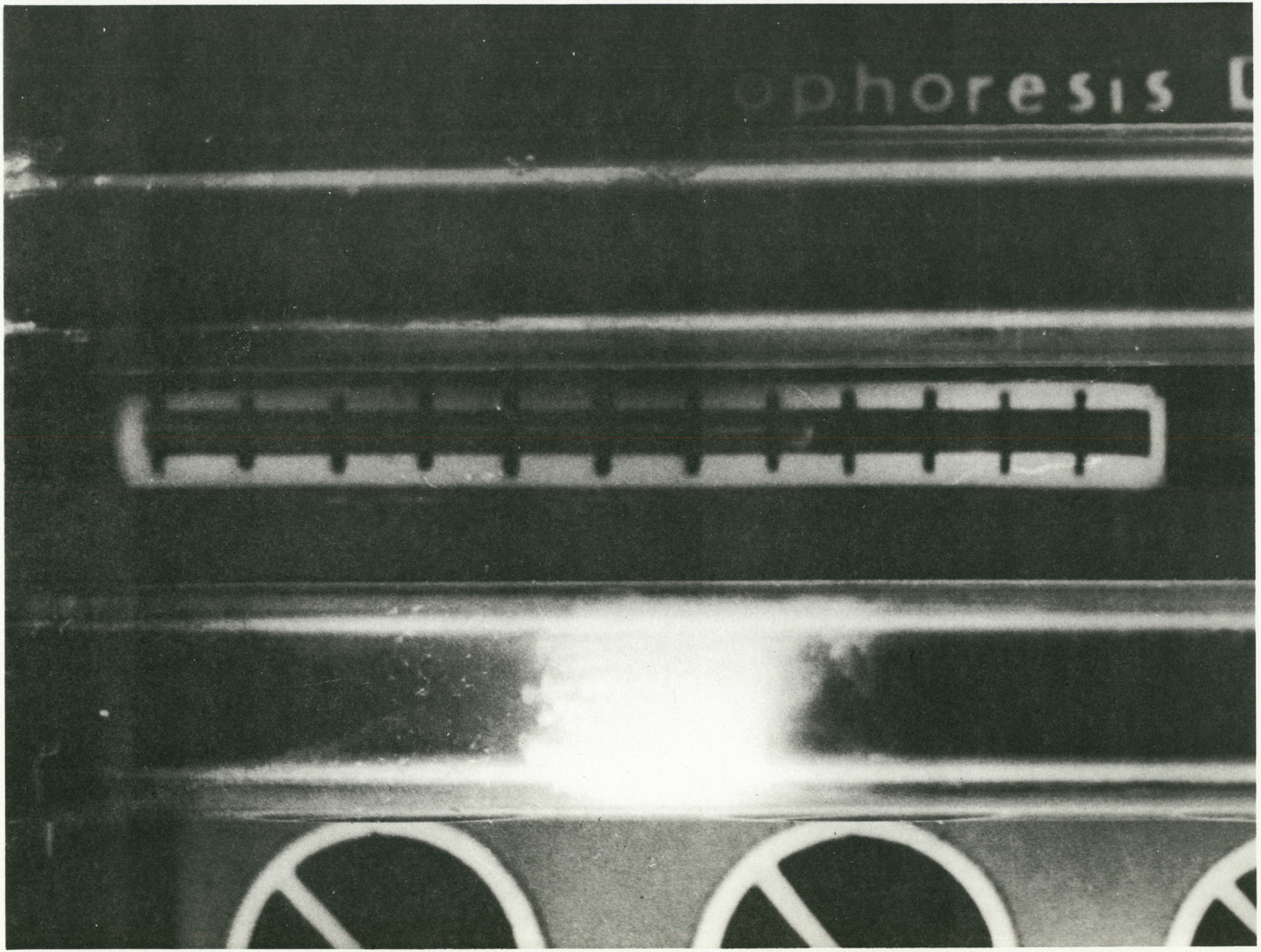

Figure 1. Streamers of Sample in a Sacrose Density Gradient

the repeatability of runs, presumably by discouraging agglomeration. It did not, however, prevent the formation of streamers.

Because of continually recurrent problems in trying to conduct experiments in Apollo 14 equipment, new cells were designed with the same interior dimensions and made from the same material. The new cells, however, were more suitable for use on earth and were water-jacketed.

Several attempts were made to separate B particles (Reference 5) from EDTA-treated human milk in the newly designed electrophoresis cells. The first such experiment was highly successful, excellent separation and good photographs being obtained (See Figure 3). Subsequent experiments, however, showed no band corresponding to the B particles. In consultation with Dr. D. H. Moore of the Institute for Medical Research we learned that he had similar experience and had just discovered that treatment of the milk with formalin is necessary for preservation of the B particles. Oddly enough, the mouse mammary cancer virus apparently does not require similar treatment. Investigation of this anomaly was clearly not within our purview, and no further experiments with milk have been performed or planned.

We also attempted to perform an electrophoresis of stained normal and leukemic human lymphocytes - with the understanding that the lymphocytes

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5. Moore, D.H., American Cancer Society Seminar, Phoenix, Ariz., April 1971.

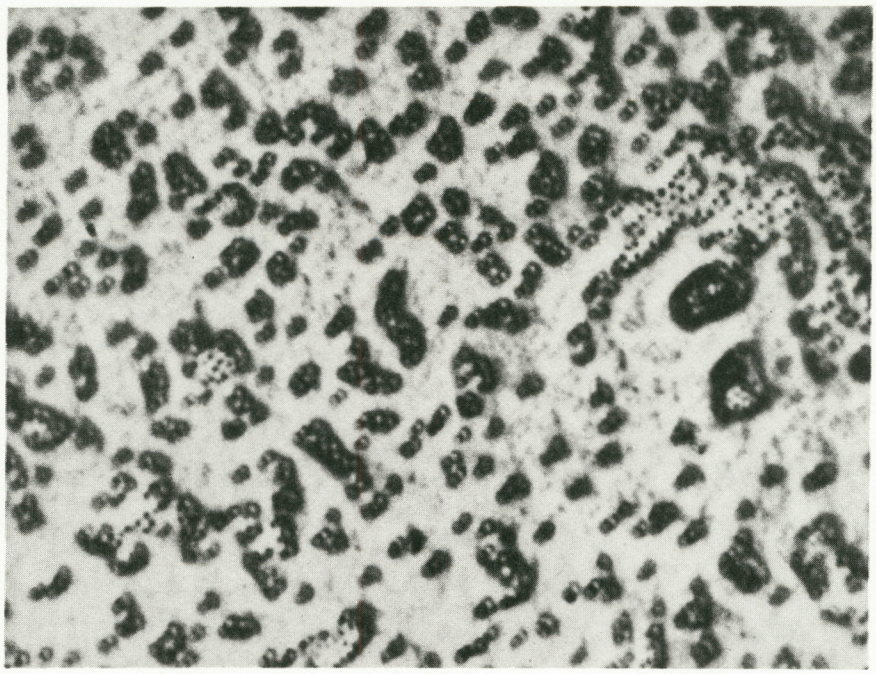

Figure 2. Agglomerated Latex Particles

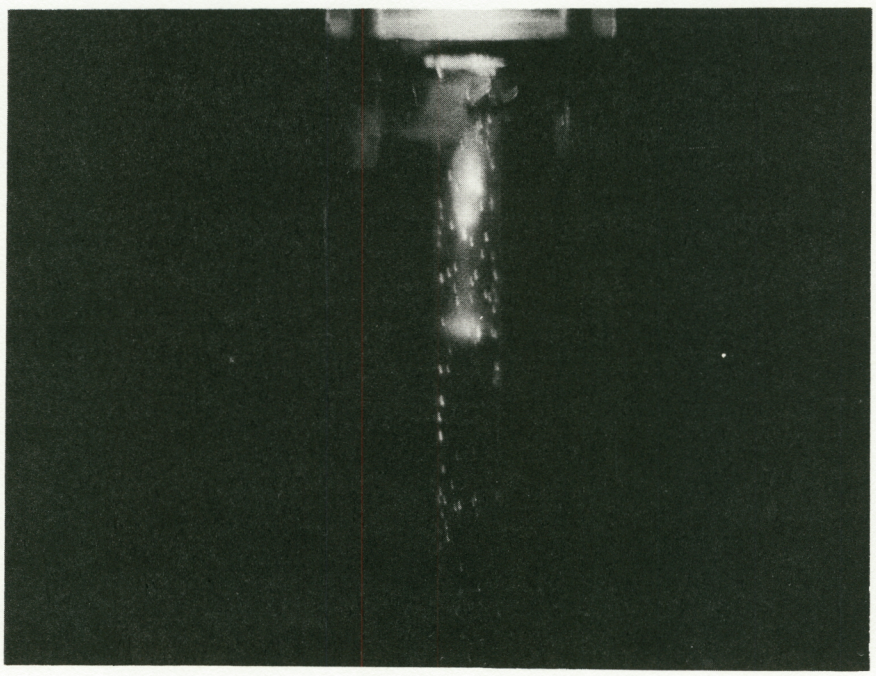

Figure 3. Electrophoretic Separation of B Particles from EDTA-Treated Human Milk

would be destroyed in the density gradient of the electrophoresis cell. However, we were unable to stain the lymphocytes with Evans blue or "Stains-All" without causing lysis.

Another aspect of the work was to determine what, if any, biologically significant separations might be attempted. The definition of such samples was undertaken by the Institute for Medical Research under a consulting agreement. An abstract of their report is included as Appendix I. The conclusions generally were that Cytochrome C would be suitable as a sample, and that no cells were known which would remain viable for the storage times required, though a human cell line, RPMI 1788, was identified which remained viable for at least six days at 23°C.

However, we were subsequently instructed that only two sizes of polystyrene latex would be considered for Apollo 16 samples (a decision reached by NASA on the advice of a USRA subcommittee), so all work with biologicals was terminated and efforts renewed to optimize conditions for separation of polystyrene latex in the new Lexan cells described above. Some fifty attempts at electrophoresis of polystyrene latexes were made, culminating in the choice of a borate buffer, pH 8.4, I = .0024, specific conductivity, $\varkappa$ = .13 mmho, with formalin and sodium lauryl sulfate added. The sample materials chosen were polystyrene latexes 0.234 and 0.79 microns diameter,

though the latter was subsequently changed in favor of an 0.8 μ sample supplied by Dr. Vanderhoff of Lehigh University. Variable results were obtained in the many electrophoresis experiments, though good separations were obtained on several occasions as exemplified in Figures 4A-4D. The electrophoresis experiments with polystyrene latex are summarized in Appendix II.

C. Investigation of Electrode Systems

Three systems were considered. These included platinum (which requires a circulating system), palladium/palladium-hydrogen, and silver/silver chloride electrodes. Each type of electrode system has its own characteristic problem. The platinum electrodes require a pump and motor with its attendant vibration; palladium electrodes may reduce some components of the buffer system; and silver chloride electrodes require the use of salt bridges.

In order to assess the desirability of changing from platinum to reversible electrodes we investigated the use of palladium and of silver chloride electrodes. Platinum electrodes were used in the Apollo 14 demonstration for the purpose of evaluating bubble removal and phase-separation systems which may be necessary in future continuous units. In relatively large continuous units, however, the pump and motor could presumably be physically

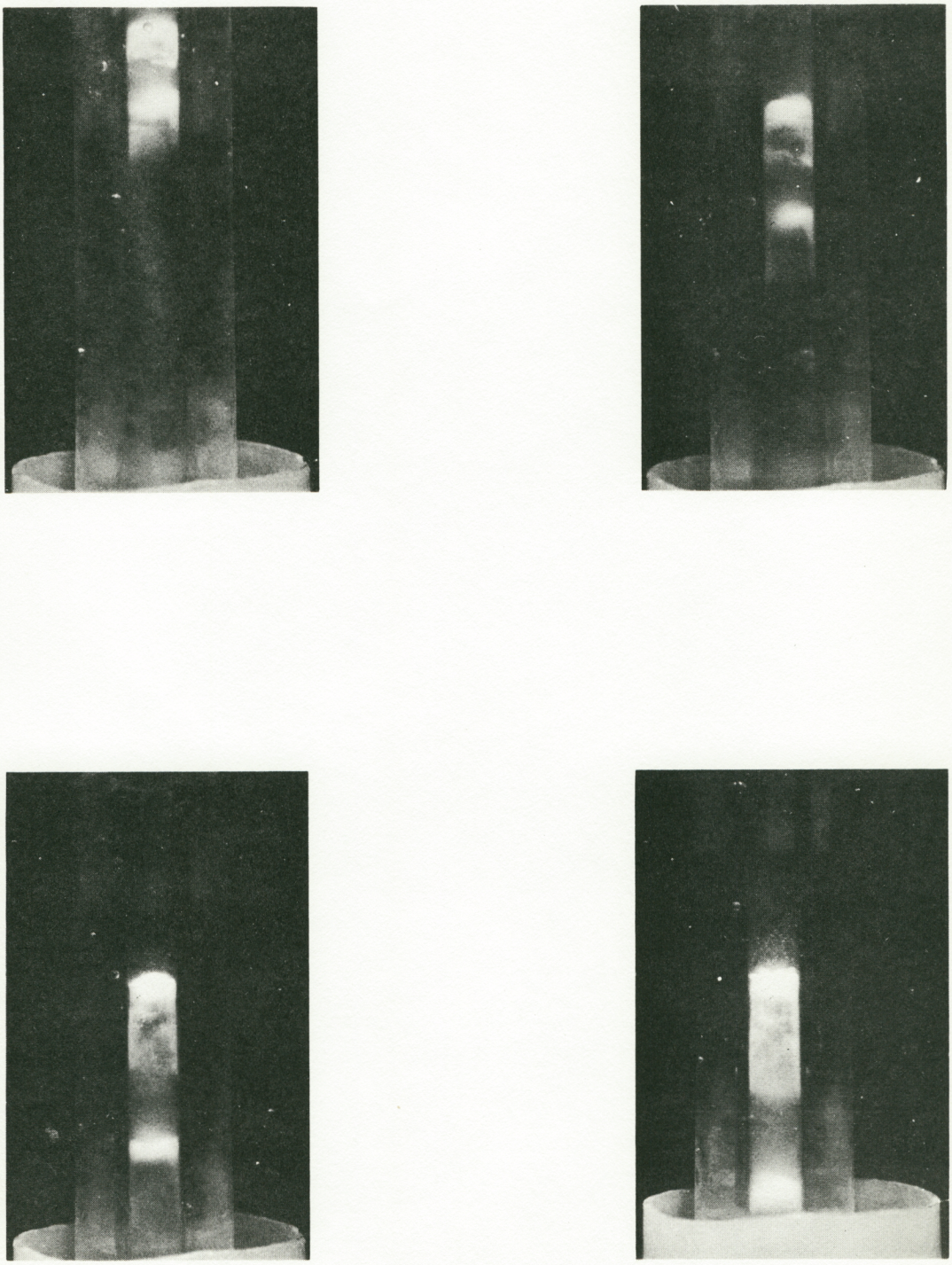

Figure 4A-D. Electrophoresis of Polystyrene Latex in a Sucrose Density Gradient.

separated from the electrophoresis cell - a condition which is impossible in Apollo-type demonstration units. The inevitable vibration in Apollo demonstration units may contribute significantly to band spreading. Thus, if reversible electrodes could be used, they would minimize disturbances in such demonstrations. Palladium/hydrogen electrodes were found to operate well in dilute (0.0015 M) tris hydrochloride buffer. No evidence was found by electron microprobe analysis of palladium contamination of the buffer after several hours operation. Unfortunately, storage tests showed that over a period of three weeks palladium/hydrogen electrodes caused a marked change in the pH of 0.0083M borate buffer containing formalin and sodium lauryl sulfate, the system selected for Apollo 16.

One of the best-known and most reliable reversible electrode systems is Ag/AgCl. In normal applications these electrodes are immersed in 1N KCl solution, and this solution is separated from other solutions in the system by a salt bridge or by virtue of its density. In our application a salt bridge would be necessary, but normal salt bridges would not be expected to survive the flight qualification vibration tests. Therefore we made salt bridges by filling, under vacuum, the pores of Porex polyethylene with a polyacrylamide gel that was made with the electrophoresis buffer. Experiments showed that

when such a salt bridge is made leak-free, it is very effective in keeping chloride ions out of the electrophoresis cell. Two such bridges were operated for 630 minutes at 2 milliamperes without evidence of chloride transport. The use of such a system on Apollo, however, is complicated by the very long storage time before flight. It would doubtless be necessary to isolate the salt bridge from the potassium chloride solution by an impervious membrane until the apparatus was to be used. Such a construction would lead to many potential leak sites as well as relatively complex start-up procedures. Therefore we decided that as long as several months of storage prior to operation was required, the safest choice of electrode system was platinum with circulating electrolyte.

III. APOLLO 16 ELECTROPHORESIS DEMONSTRATION APPARATUS

A. Description

Incorporating improvements suggested by our experience with the Apollo 14 demonstration, a similar unit was flown on Apollo 16. The changes included an enlarged window, for better visualization of the experiment, the elimination of the electromagnetic interference screen (since this was shown to be unnecessary although the test that showed it unnecessary were carried out too late to incorporate this improvement on the Apollo 14 unit) and lighter construction of the electrophoresis cell to improve visibility. This included a change from a solid plastic block with holes drilled in it to thin-walled plastic tubing with machined headers. A number of measurement devices were also included such as an Accutron watch, in order to confirm the interval between photographs although these were taken automatically; milliameters-one for each tube to indicate a possible change in the current flowing through each of the 3 electrophoresis cells or perhaps to indicate that one of the 3 cells was not operating; markings on the electrophoresis tubes 1 cm apart in order to allow calibration of the rate of movement; and a thermometer to indicate the ambient temperature at the cell. A change in the sample release mechanism was made necessary and desirable because this was the one mechanical failure that occurred on Apollo 14. Therefore considerable effort was devoted to the development of a release mechanism which would be highly reliable and would cause minimum disturbance to the samples when operated. We found that a

Kapton film 0.05 mm thick could be withdrawn smoothly from the cell without causing disturbance of the fluid. This was easily accomplished with a knob-actuated roller. The Apollo 16 device consisted of 3 electrophoresis cells about 10 cm long by 0.6 cm inside diameter filled with a borate buffer containing 0.02% sodium lauryl sulfate and 0.1% formalin. The sodium lauryl sulfate was included in order to keep the polystyrene latex samples from agglomerating. The formalin was included in order to inhibit any bacterial growth which might occur. The buffer had a pH 8.4, ionic strength 0.0024. Both ends of the electrophoresis cells were closed with cellulose acetate/nitrate membranes. The samples were contained in a volume 1.6 mm by 4.7 mm diameter and were retained between the cathode membrane and a Kapton film. Platinum electrodes were used, and the electrolyte was circulated over them by a peristaltic pump. The electrolyte was interchanged between the cathode and anode to minimize pH changes. Because of the requirement for a 3 1/2 month storage, the same buffer was used in the electrophoresis cells as was used in the electrode compartments, instead of using more concentrated solution in the electrodes as is frequently done. The system would have been simpler if reversible electrodes could have been used, but we could not depend on salt bridges for the long storage time required and palladium/hydrogen electrodes proved unstable during prolonged storage.

Photographs of the unit are shown as Figures 5 and 6. The outer casing was made of blue anodized aluminum and measured about 10 x 12.7 x 17.8 cm plus the switches, knobs and camera tripod mounting blocks. It weighed about 3.28 Kg and consumed about 30 watts.

B. Mock-up Unit

A mock-up of such a demonstration unit was constructed and shown to the COR and representatives of Quality Control at NASA-MSFC on 19 October 1971. Minor changes were suggested in the construction of the camera mount as well as some changes in materials and components used in the apparatus. The following week at a Critical Design Review some further changes in the camera mount were suggested. These changes were incorporated in the drawings and preparations made for construction of a training unit according to the revised drawings.

C. Training Unit

A training unit was delivered on 15 November 1971. By agreement with the COR and others, reached at the Critical Design Review, the training model was not operable. In lieu of an operable model - which was deemed useful only if it could be run repeatedly - a training manual was supplied which contained detailed operating instructions, an explanation of the purpose and significance of the demonstration, and photographs of the electrophoresis of polystyrene latexes as performed on earth in a density gradient. A copy of the training manual is attached as Appendix III.

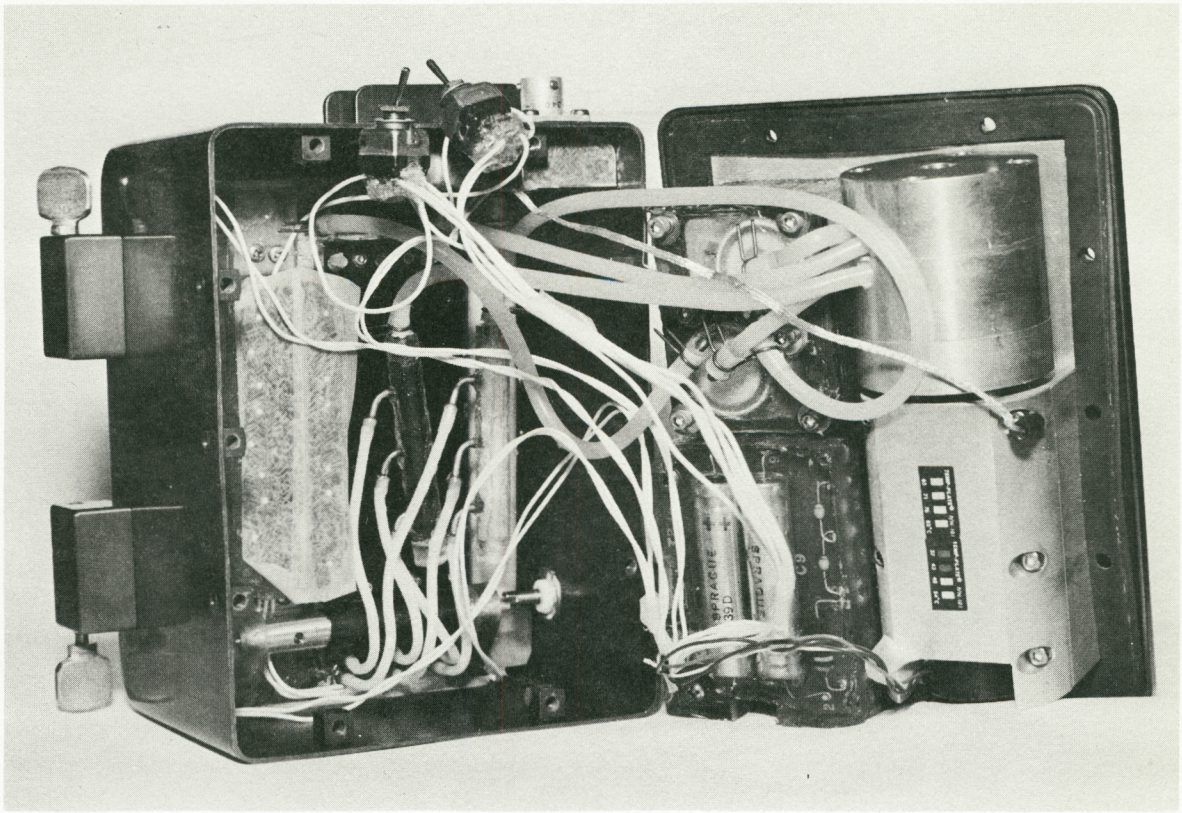

Figure 5. Interior of Apollo 16 Electrophoresis Demonstration Unit.

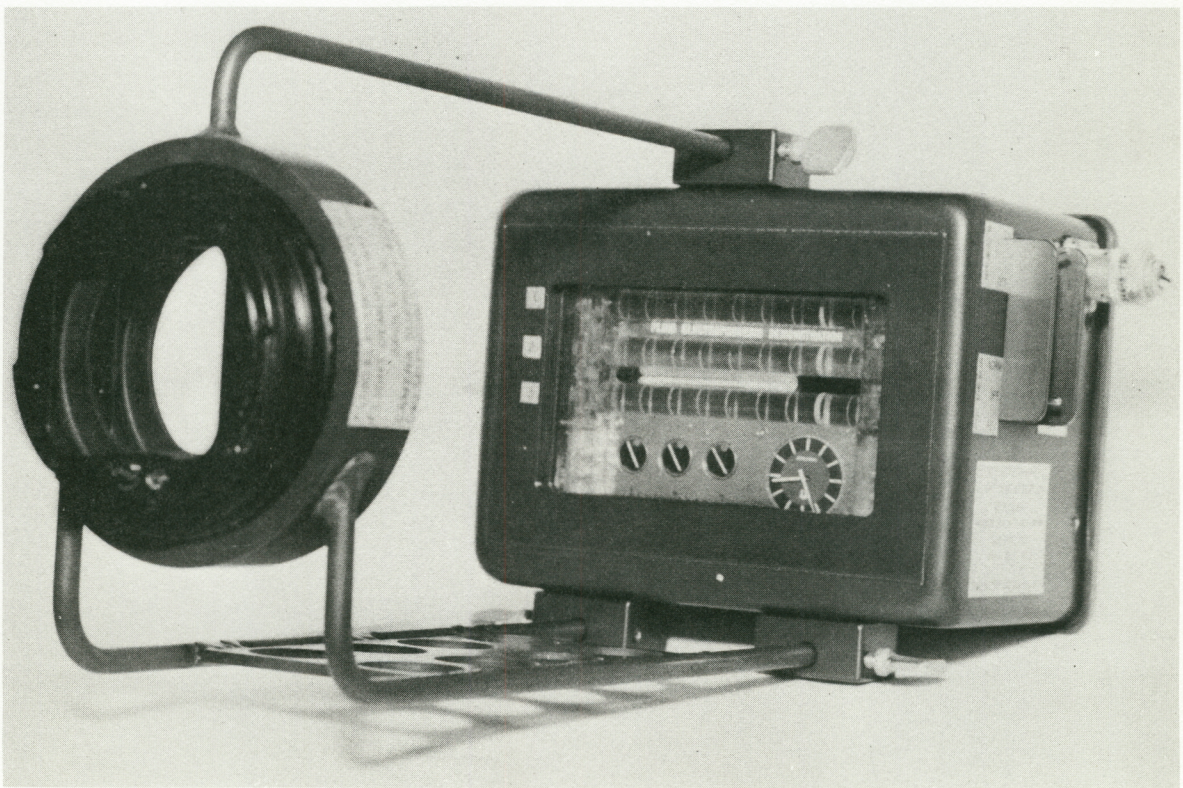

Figure 6. Exterior of Apollo 16 Demonstration Unit.

D. Photography

A 70 mm Hasselblad 500 EL camera was obtained for photographic studies of the experiment. After trying several combinations of lenses, extension tubes, and added close-up lenses, we determined that the most satisfactory arrangement involved the use of two Hasselblad M21 extension tubes mounted on the experiment tripod. The camera body was attached to the outer end of the extension tubes, and a Zeiss Planar f.2.8/80 mm lens to the inner end. With Ektachrome MS film and this lens arrangement, good exposures were obtained at settings of 1/15 second, f 2.8, or equivalent.

A meeting was held at MSC to confirm stowage and photographic requirements. A class III NASA Hasselblad camera was attached to the demonstration tripod, and it was immediately evident that there is enough difference between the NASA cameras and those sold commercially that changes would have to be made in the camera mount. It was also suggested by Mr. N. Lamar, MSC, that type 3401 black and white film would provide more reliable data than the planned SO 368 or 168. Accordingly, trial exposures were made with type 3401 film in our Hasselblad camera. The best exposure appeared to be 1/30 second at f8. Some exposed film was also sent to Mr. Lamar for evaluation at MSC.

E. Qualification Unit #003

Following completion of the training unit, a qualification unit, serial 003, was built and acceptance tested in accordance with the Apollo 16

Electrophoresis Demonstration Acceptance Test Procedures and Amendment I to Contract NAS 8-27797. The unit was shipped to NASA/MSFC on 29 November 1971.

On 10 December 1971, the qualification unit, serial 003, was received from NASA-Marshall Space Flight Center for repair of fluid leakage. The unit was opened in the presence of Mr. C. Seaver, S&E-Qual-ATA, and a determination made that the flexible Viton tubing was crimped. The tubing was replaced, the unit closed and operated for a brief period. The new tubing began to leak after about a half-hour's operation. This made it evident that the unit could not operate with the specified Viton tubing, and we were allowed to replace it with silicone and Teflon tubing of the type used in the Apollo 14 electrophoresis demonstration. Other repairs were also made, mostly incidental to the fluid leakage.

Subsequently, testing revealed the need for better attachment of the thermometer and minor changes in the phase separators. A number of other minor changes and improvements were made as a result of acceptance tests.

Again the unit passed the specified acceptance tests at General Electric, only to experience leakage when tested at NASA/MSFC. Finally we learned that the tests at MSFC included a 5 psia soak and operation, tests which were not required for acceptance. The leakage problem was then traced to a pinhole leak in the cathode header. This was repaired and the unit resubmitted for testing at MSFC. Meanwhile it was decided that this

unit should be used as a training unit. It was used in this capacity until the samples finally agglomerated as shown in Figure 7.

F. Contamination Control

Because of the bacterial contamination problem encountered in the Apollo 14 demonstration, a plan was developed for contamination control and sterilization of the Apollo 16 flight and flight back-up units. The contamination control and sterilization plan is included as Appendix IV. Some parts of this plan were not carried out since, as described below, the units finally assembled for flight and back-up were not sterilized because of stress-cracking problems.

G. Flight Back-up Unit #004

The flight back-up unit, serial 004, was sterilized and aseptically assembled according to the procedures outlined in Appendix IV. It passed the acceptance tests at General Electric and at MSFC between February 7 and 9, 1972. It then passed qualification testing at MSFC (unit 003 having been redesignated as a training unit). Early in March cracks were found in Lexan parts in both the phase separator and the electrophoresis block. This failure, together with the January 20 failure of Lexan in unit 003 (which had been attributed to freezing during shipment) indicated that some basic problem existed in the design of parts and/or choice of materials.

An extensive investigation of the problem was undertaken with the help of Dr. R. Snyder, COR, A. Krupnick, and C. Seaver of MSFC, and D. Caird, GE Lexan specialist. Mr. Caird's report is included as Appendix V.

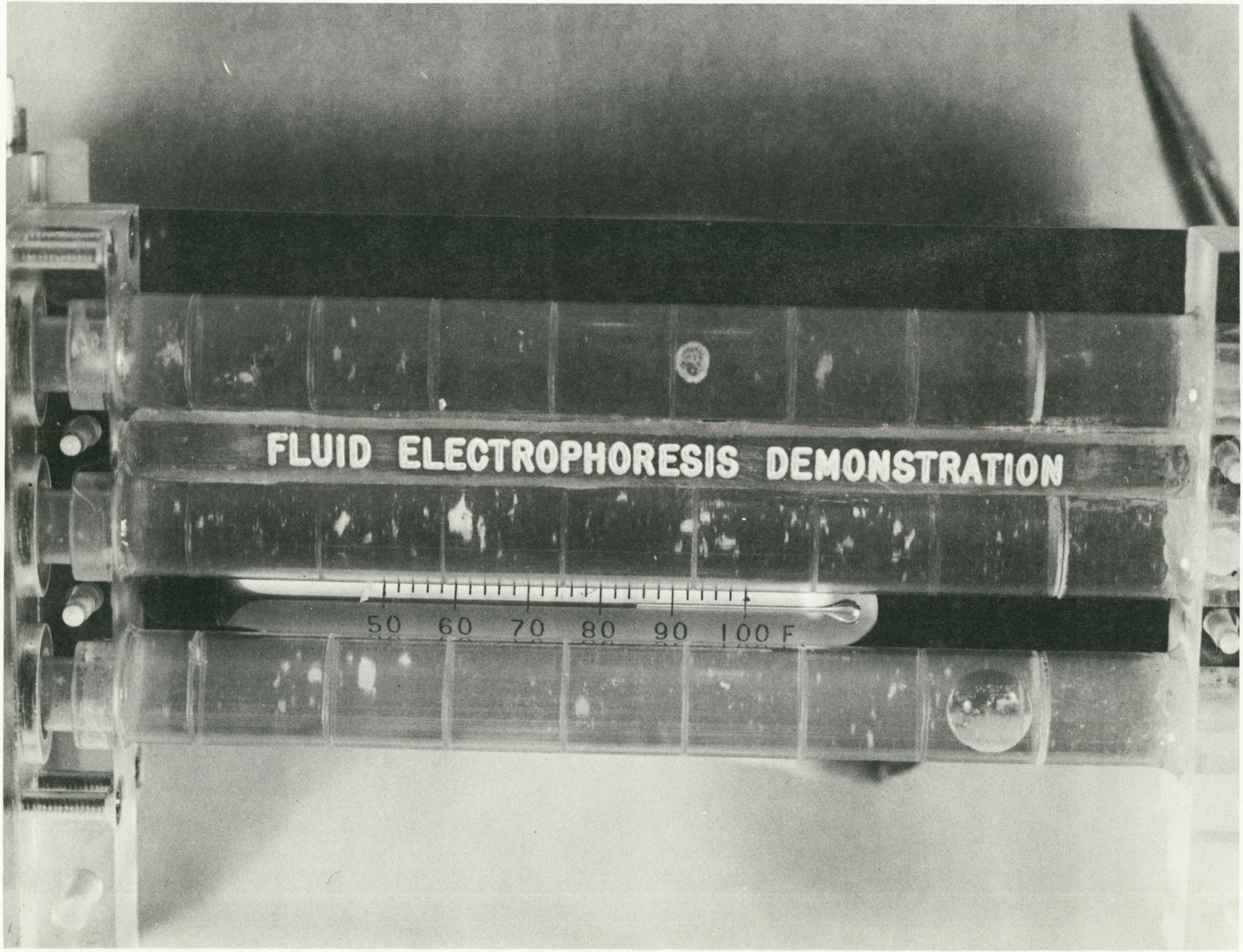

Figure 7. Agglomerated Samples in Training Unit After Repeated Use

Subsequent testing has borne out most of Mr. Caird's conclusions, even to identification of the Nephlex membrane as a probable source of stress. It was found that these membranes expand 15-20% after soaking in water. Stress from this source was subsequently relieved by incorporating an RTV 168 cushion around the membrane. Numerous tests were performed on specimens machined by various procedures to simulate crack-sensitive parts of the electrophoresis cell and subjected to various post-treatments. The tests quite clearly showed that careful machining with sharp tools using only water as a coolant produced the best results. Annealing was shown necessary for the best results, and a double annealing with intermediate remachining of close tolerance parts was found to be the best. Some of the test pieces are shown in Figure 8. Sixteen tests were started at the same time in which four-point bending specimens under stress were immersed in buffer. Some of these samples were as-machined, some were annealed, some were sterilized, and some were conformal-coated with RTV 168. None of the specimens have broken three months later.

Nevertheless, unit 004 was reassembled with non-annealed parts. The sterilization procedure was omitted in the hope of minimizing stress-cracking influences, and a conformal coating of RTV 168 was added. The unit was vacuum tested at 5 psia for six hours, and was cycled ten times at five minute intervals between 5 psia and atmospheric pressure. This was

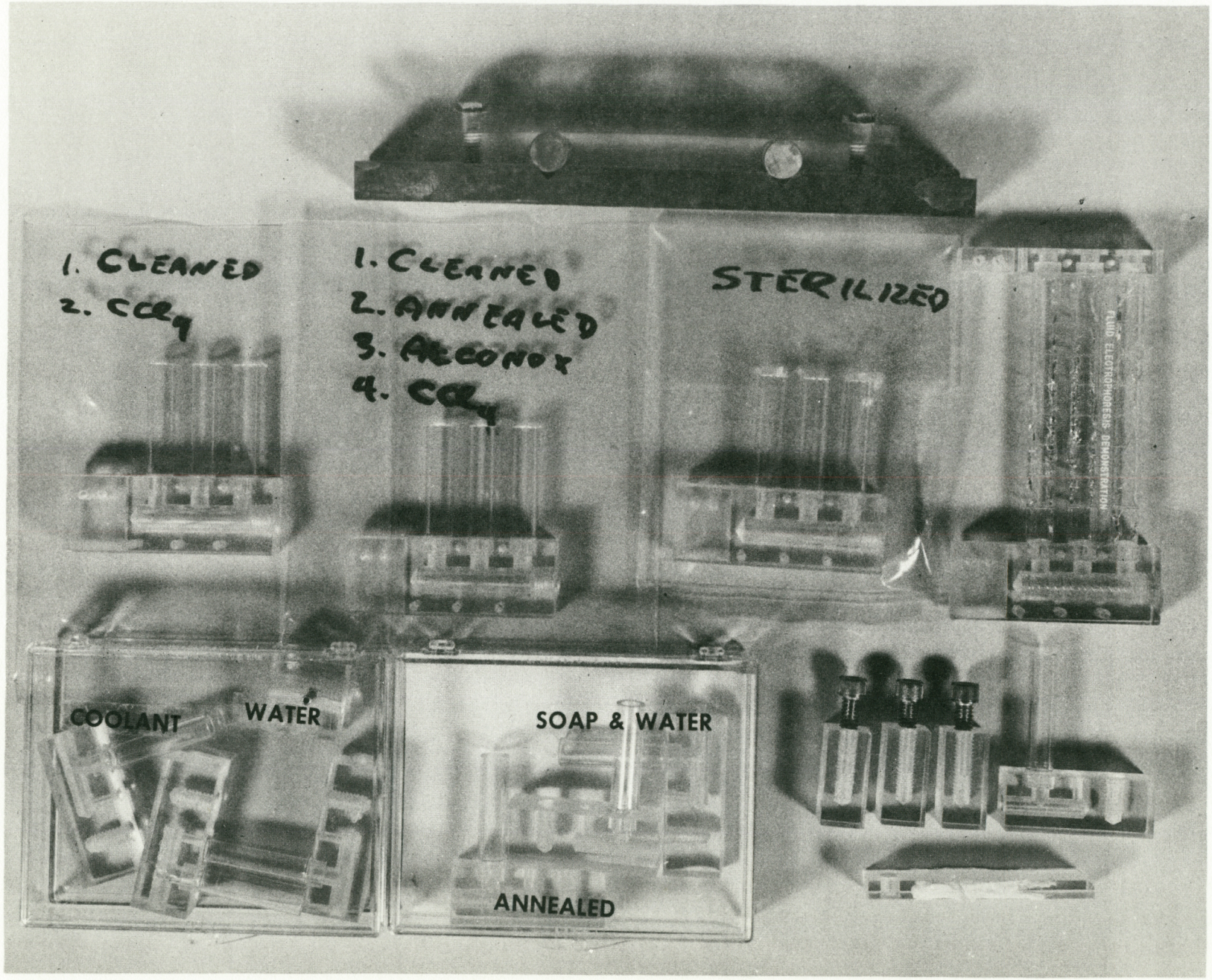

Figure 8. Test Specimens for Stress-Cracking Experiments

done because of an acceptance review scheduled for March 15; and as expected, failure of the Lexan was again observed about three weeks later. Sterilization of this and all subsequent units was omitted by direction of the COR.

The unit was again repaired early in April, this time with parts made according to Mr. Caird's recommendations. Because of all the problems with stress-cracking we decided not to coat the inside of the electrophoresis tubes with collodion. While we had no direct evidence that the coating contributed to the stress-cracking problem, it is applied in an ether/methyl cellosolve solvent which we felt might be deleterious. There was some evidence to indicate that omission of the coating would have little effect on the experiment, and we felt that a slight loss of sharpness of the sample bands was preferable to possible loss of the entire experiment. Paired electrophoresis runs in the modified Apollo 14 block mentioned earlier had shown little or no difference between cells coated with collodion and those with plain Lexan walls. A photograph of one such experiment is shown in Figure 9. More refined measurements made since the Apollo 16 flight in a Beckman CPE have shown that there is a slight difference in the wall zeta potentials, though electroosmosis is not a serious problem in either case. Data from the CPE indicate that the electroosmotic flow in the center of the tubes of the flight experiment was about 0.0125 cm/sec. and would have been about 0.006 cm/sec. with a collodion coating. Photographs of the CPE experiments are shown in Figures 10 and 11. The unit passed acceptance tests and was flown on Apollo 16.

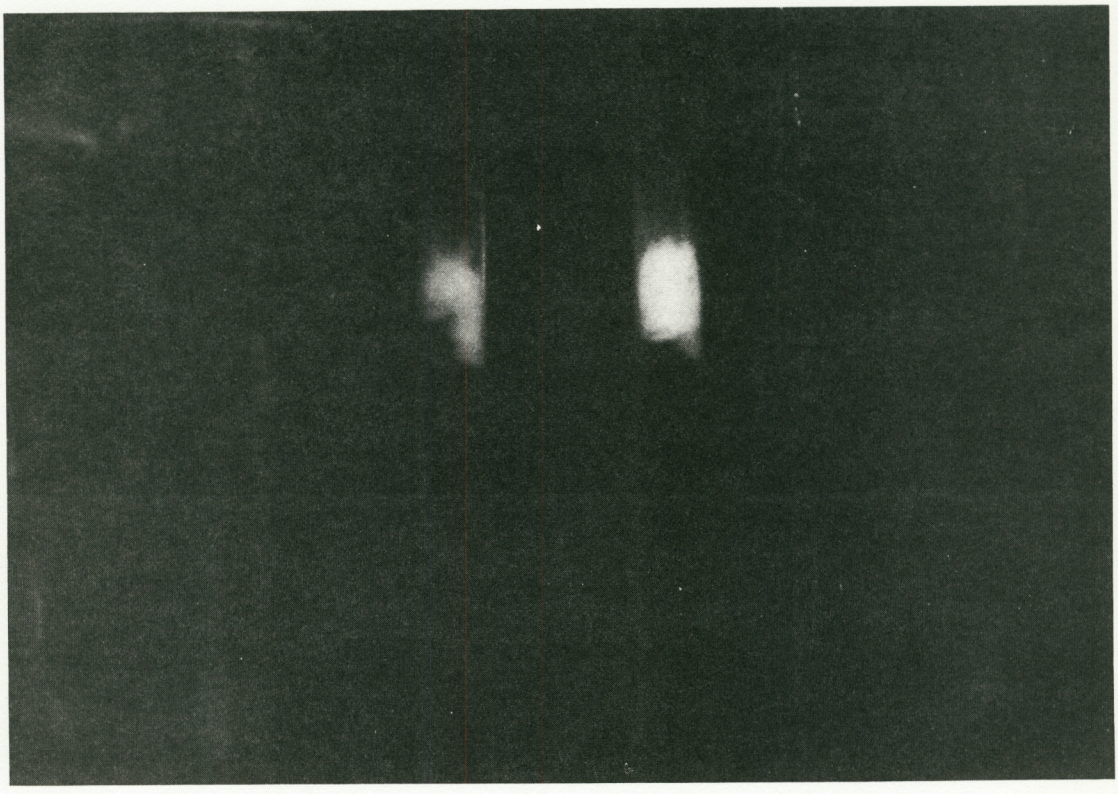

Figure 9. Paired Electrophoresis Experiments with Coated and Uncoated Cell Walls.

Tube on the left is Lexan. Tube on the right is Lexan coated with collodion.

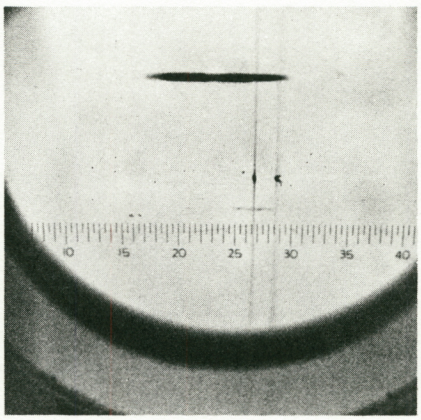

Figure 10. Electrophoresis of Polystyrene Latex (0.234 and 0.8 micron) in the Beckman CPE with Collodion-Coated Walls.

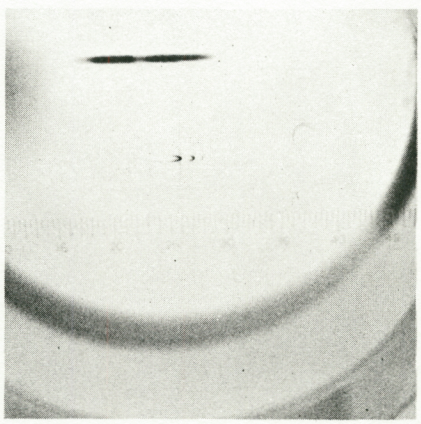

Figure 11. Electrophoresis of Polystyrene Latex (0.234, 0.8 and 2.0 micron) in the Beckmen CPE with Lexan-Coated Walls.

H. Flight Unit #005

The history of the flight unit, serial 005, closely parallels that of unit 004. It was assembled during the latter part of December 1971, and passed all tests. Stress-cracking failure was observed March 1, 1972. The unit was rebuilt with properly machined, annealed parts and accepted on April 5, 1972. About a week later, bubbles were observed in all three tubes, "anomolous particles" were observed in the middle tube, and "marginal" (though acceptable) insulation resistance was measured. The unit was returned from KSC to MSFC for analysis.

IV. APOLLO 16 ELECTROPHORESIS DEMONSTRATION EXPERIMENT

A. Background

Traditionally, gravity and its effects have profoundly affected the electrophoresis process. In many ways this effect has not always been negative. For example, the use of supports such as paper or gels was originally intended to combat the convective effects of gravity. But the subsequent development of these techniques has led to vast improvements in resolution as is typified by disc electrophoresis.

Fluid or free electrophoresis is becoming ever more important in spite of the inherently lower resolution than can be obtained by some of the more sophisticated zone methods. This is due largely to the increasing importance of the separation of viable cells which cannot migrate through supporting media. In the field of fluid electrophoresis efforts to defeat the effects of gravity have led to a number of ingeneous solutions including magnetically-driven flow in convoluted tubes, anular cells between rotating horizontal cylinders, and horizontally flowing density gradients. The most widely used form of free electrophoresis, however, remains the vertically flowing film of fluid with a horizontally applied field. But even in this equipment the effects of gravity are felt. In order to minimize convective disturbances the film of fluid must be kept thin-on the order of 0.5 to 1.5 mm. Inevitably this means that the sample stream is close to the walls of the cell. The fluid/wall interface

is a discontinuity, and except in special cases discontinuities are to be avoided in any electrophoresis. (Exceptions are, of course, the intentional pore size discontinuities in disk electrophoresis and the ionic species discontinuities in isotachophoresis). The combination of Poiseuille flow patterns and electroosmotic disturbances is probably the major factor limiting the resolution attainable in present day free flow electrophoresis equipment. Strickler's elegant work on the control of cell wall zeta potential and his more recent work (Reference 6) on the use of split fields with different wall zeta potentials has made it possible to sharpen the resolution of bands in any one desired product fraction. But as yet there exists no method for achieving high resolution simultaneously in all product fractions. It seems likely that high resolution could be attained if a relatively thick cell could be used. This should be possible if the equipment were to be operated in an orbiting space vehicle where gravity is essentially absent. It is primarily for this reason that we have been interested in the development of fluid electrophoresis equipment for use in space.

The first step in this equipment development was taken in 1971, when a small demonstration unit was flown on Apollo 14. Due to mechanical,

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6. Stricker, A. and Sacks, T., "Focusing in Continuous Flow Electrophoresis Systems by Electrical Control of Effective Cell Wall Zeta Potentials", presented at the New York Academy of Sciences Conference in Isoelectric Focusing and Isotachophoresis, New York, May 1972.

photographic, and bacteriological problems, this demonstration, while it did work, was not completely successful. It did, however, demonstrate the successful operation of several sub-systems such as that which separates electrolysis products from the recirculating electrolyte.

B. Apollo 16 Demonstration

Incorporating improvements suggested by the results of the Apollo 14 demonstration, a second unit was flown in the Spring of 1972 on Apollo 16. The principal objective of this experiment was to demonstrate that cohesive bands of sample can be caused to migrate electrophoretically through a fluid in a manner which would be impossible under similar conditions here-on-earth. Figures 12A-D depict the effect of attempting to electrophorese polystyrene latex in a horizontal tube. While it is unlikely that anyone would attempt to perform an electrophoresis in this manner, nevertheless the analogy between the obvious convection and sedimentation in Figure 12 and the problems that are commonly encountered in fluid electrophoresis on earth is obvious. By comparison, the same experiment performed on Apollo 16, as shown in Figures 13A-D, demonstrated a relatively sharp sample band. The time after sample insertion is the same in each of the matched pictures. Figures 12 and 13A were taken 40 seconds after experiment activation; 12B and 13B at 120 seconds; 12C and 13C at 160 seconds; and 12D and 13D at 200 seconds.

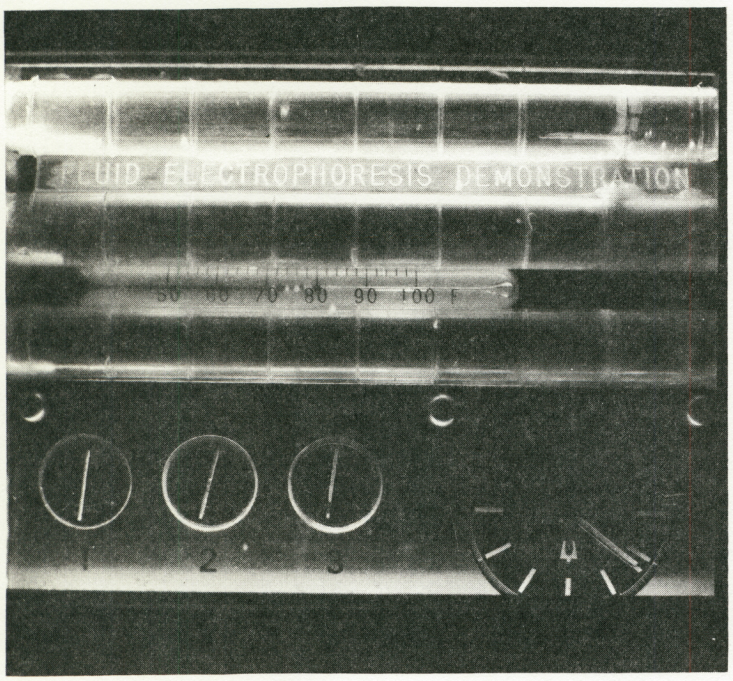


Figure 12A

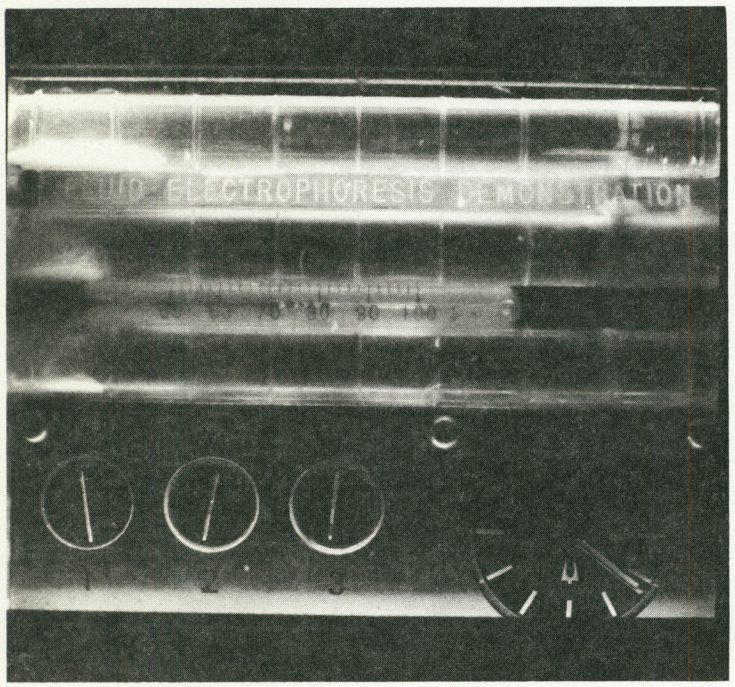


Figure 12B

FIGURE 12. ELECTROPHORESIS OF POLYSTYRENE LATEX ON EARTH

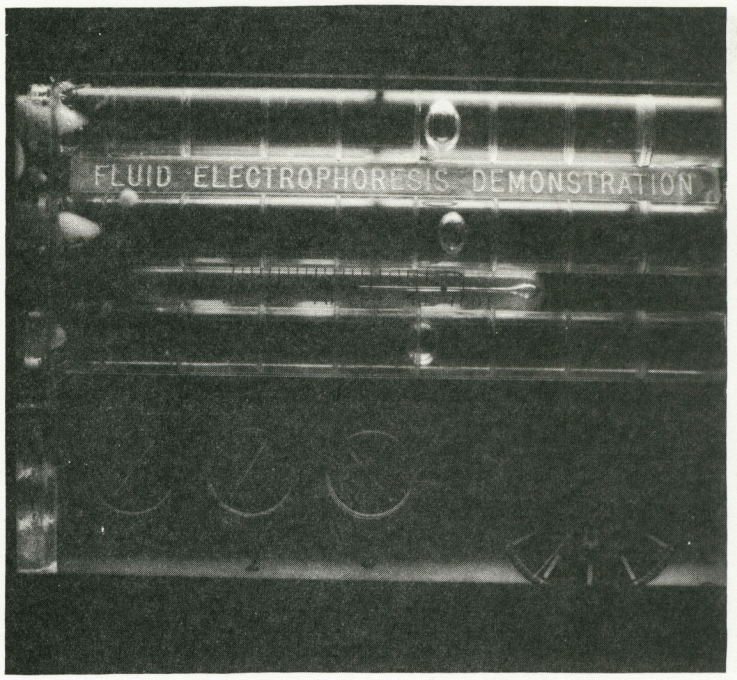


Figure 13A

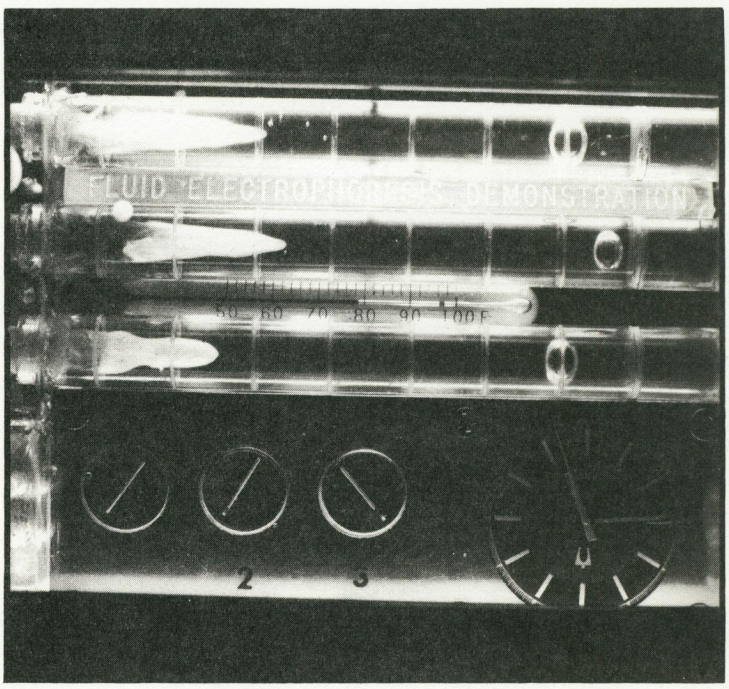


Figure 13B

FIGURE 13. ELECTROPHORESIS OF POLYSTYRENE LATEX ON APOLLO 16

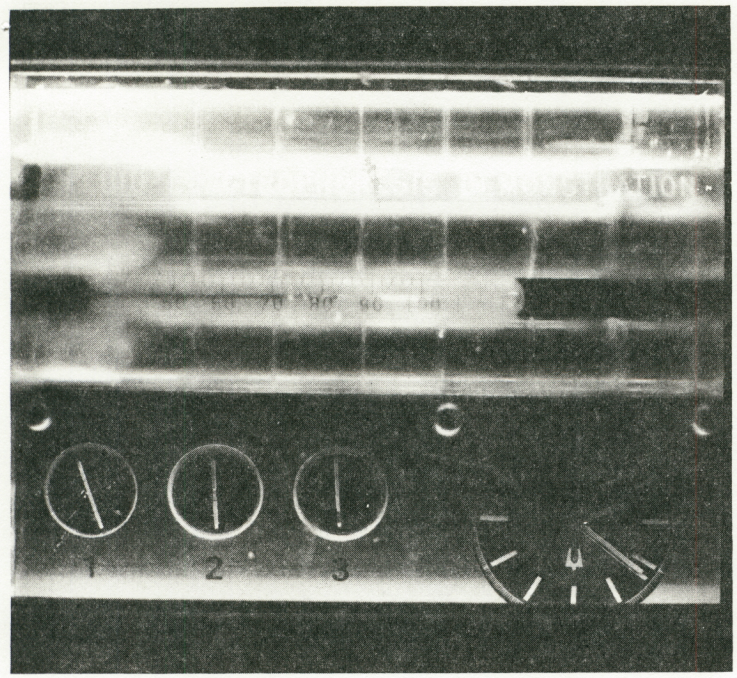


Figure 12C

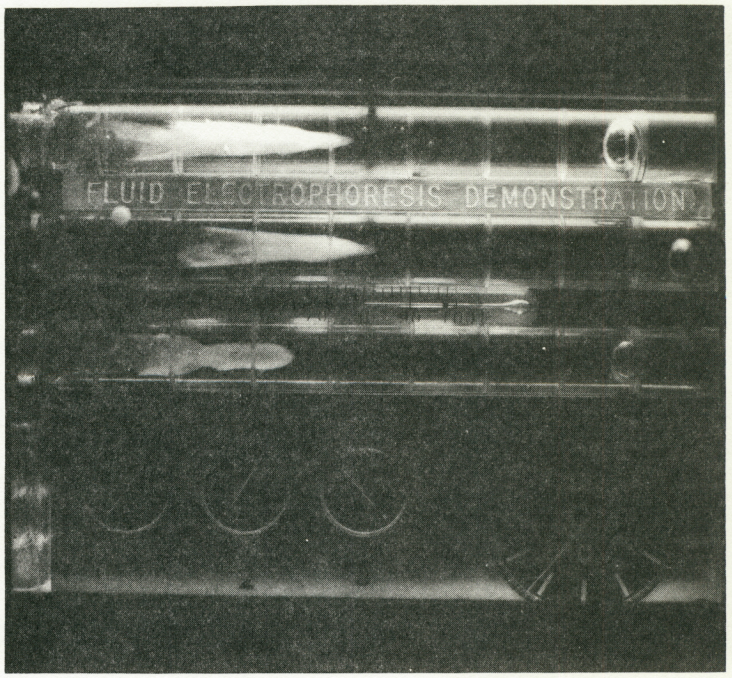


Figure 13C

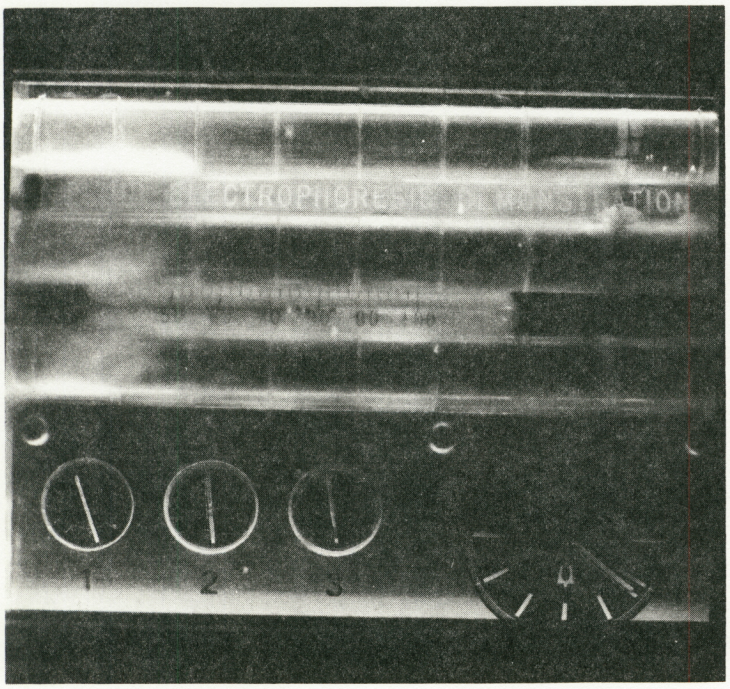


Figure 12D

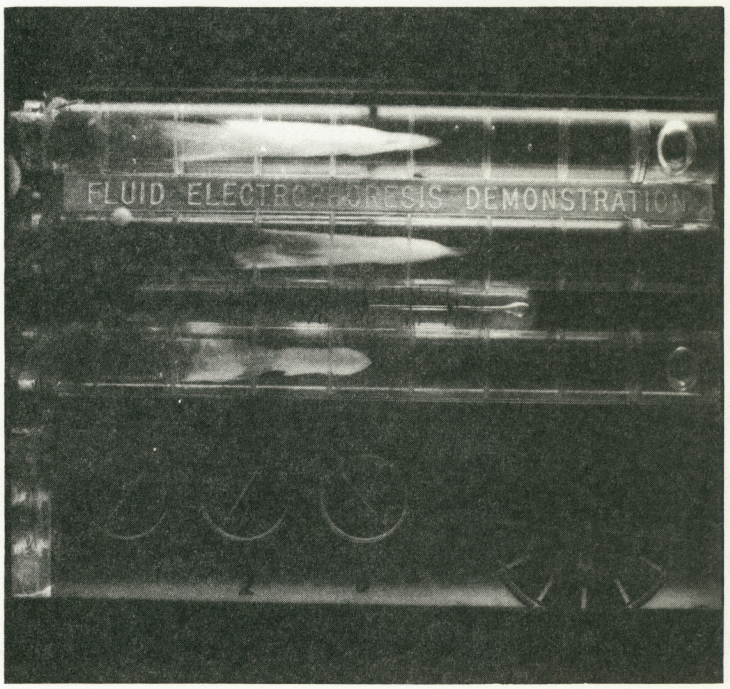


Figure 13D

FIGURE 12. ELECTROPHORESIS OF POLYSTYRENE LATEX ON EARTH

FIGURE 13. ELECTROPHORESIS OF POLYSTYRENE LATEX ON APOLLO 16

The band is not as sharp as we had anticipated, but inspection of Figure 13 indicates a relatively compact band containing most of the sample with a skirt or trail following it. The leading edge (i.e. right hand edge) of the sample band in the lower tube in Figure 13 has migrated a distance of about 5 cm from the original position.

The experiment was activated by withdrawal of the Kapton membrane, in effect admitting the samples into the electrophoresis tubes where they were subject to an electric field of approximately 29 volts per centimeter.

The samples were 0.2 micron and 0.8 micron polystyrene latexes, a mixture in the upper tube of Figures 12 and 13, the 0.8 micron particles in the center tube, and the 0.2 micron particles in the lower tube. One of the purposes of this particular set of 3 experiments, suggested by Dr. C. J. VanOss, a USRA consultant to NASA, was to determine whether any interaction might occur in the mixed samples. As can be seen, the leading edge of the band in the center tube is slightly ahead of that in the upper tube and while the difference is small it is in the direction expected if the slower 0.2 micron particles did impede the progress of the faster 0.8 micron particles.

The shape of the leading edge of the sample in the lower tube of Figure 13 is interesting in that it is not parabolic, but pointed. This effect was to be expected as a sum of parabolic distribution due to electroosmotic flow and a temperature profile across the tube, being hottest at the center. The additional "nose" on the front of the upper and middle tubes is a very small fraction of the total sample, and may represent an inhomogeneity in the 0.8 micron particles. The trailing part of the band in the lower tube is essentially a hollow cylinder whose outside edge is approximately at the point of zero electroosmotic flow.

The trailing portion of the bands developed originally almost at the start of the experiment. Its cause is uncertain, though it has been variously attributed to electroosmotic spreading of the bands; a wake effect caused by passage of the sample through the tube; a wall effect which may be merely the fact that in a "pre-packaged" system some of the sample must be adjacent to a wall with consequent interaction; or it may involve contamination of some of the latex particles from storage in polycarbonate in the presence of other materials such as Kel-F grease. It has recently been brought to our attention that polystyrene latex is extremely sensitive to trace contaminants. (Ref. 7). The use of demineralized water in the buffer can lead to noticeable trailing of latex bands as observed in the ground-based tests with a Beckman Instruments Company CPE, while under the same conditions extremely sharp bands are

obtained if the buffer is made with distilled water. Ground-based experiments with a Beckman continuous particle electrophoresis (CPE) instrument indicate mobility value of 4.3×10^{-4} $cm^2/v.sec.$ for the 0.2 micron latex, 6.0×10^{-4} $cm^2/v.sec.$ for the 0.8 micron latex, and an electroosmotic flow in the center of the polycarbonate tube of 0.0125 cm/sec. under the conditions of this experiment. These results are in almost exact agreement with the values obtained in the flight experiment where apparent mobilities of 8.6×10^{-4} $cm^2/v.sec.$ and $10.4 \pm 0.1 \times 10^{-4}$ $cm^2/v.sec.$ were observed for the 0.2 and 0.8 micron samples respectively. The expected electroosmotic flow is equivalent to an apparent mobility of 4.3×10^{-4} $cm^2/v.sec.$ giving net electrophoretic mobilities of 4.3×10^{-4} $cm^2/v.sec.$ for the 0.2 micron sample and 6.1×10^{-4} $cm^2/v.sec.$ for the 0.8 micron particles (Reference 8).

Additional confirmation of these figures can be obtained from consideration of the rate of movement of the trailing part of the sample which is very close to the point of zero electroosmosis (at a distance 0.707r from the tube center). The trailing edge of the 0.2 micron sample in the lower tube of Figure 8 moved at a rate equivalent to a mobility of 4.25×10^{-4} $cm^2/v.sec.$, while the trailing edge of the 0.8 micron sample (in the center tube) moved at a rate equivalent to a mobility of 5.75×10^{-4} $cm^2/v.sec.$

Reference 7. Sacks, T., Beckman Instrument Co., private communication.

Reference 8. Jones, T., Beckman Instruments Inc., CPE Exchange #5.

C. Conclusion

While it does not appear feasible to determine the cause of the observed trailing, the lack of lateral diffusion or disturbance of the samples is encouraging in that it indicates that with proper sample injection something akin to zone electrophoresis in free fluid should be possible in the absence of gravity. More important, it indicates that in the absence of gravity it should be possible to electrophorese viable cells even at very low voltage gradients as would be required if the separation were to be done in isotonic salt solution. There would be no need to perform the separation rapidly with high voltages in weakly ionic solutions as is the current practice since the cells in isotonic salt solution would remain viable for prolonged periods and, of course, would not sediment or diffuse significantly.

APPENDIX I

INSTITUTE FOR MEDICAL RESEARCH

REPORT ON ELECTROPHORESIS STUDY

A large number of electrophoretic runs were carried out in the GE Apollo 14 apparatus in order to determine: (1) whether or not the performance of the cell depended primarily upon heating effects that could be expected to be unimportant in a zero-gravity field and (2) what would be a proper standard material to use as a demonstration in Apollo 16 or later flights.

1 - The initial results at various current densities in a sucrose density gradient prepared in barbital buffer, pH 8.6, ionic strength = 0.0375 showed that even at current densities as low as 2 milliamps/cell (6 milliamps applied to the 3-cell apparatus) convection cells were formed due to differential heating effects. These convection cells destroyed the electrophoretic boundaries before migration to the center of the column.

2 - It was shown that convection was the prime source of trouble by establishing a polyacrylamide column in the apparatus. Since convection effects were eliminated in the gel, sharp electrophoretic fronts

were obtained even at 7 milliamps/cell (21 milliamps for the 3-cell apparatus). This was the maximum allowable current input since above this, the gel boiled.

These experiments indicate that the prime problem with large-scale free electrophoresis is, indeed, density inequalities due to heating effects. Paradoxically, cooling the apparatus (using a modified jacketed apparatus supplied by GE) actually intensified convection effects because of the enhanced wall-to-center temperature differential in the electrophoretic column. These results would seem to indicate that, in a zero-gravity field where density differentials due to heat should not be troublesome, the main problem of large-scale free electrophoresis might be overcome.

3 - Limited experiments with dyes and proteins in gel columns established in the apparatus indicated:

a) simple dyes migrate well but do not yield sharply demarcated boundaries due to diffusion (2 milliamps/cell. 25°C, 3 hr run; buffer as above). Human hemoglobin gives multiple fronts due, possibly,

to decomposition to methhemoglobin under the same conditions.

b) Cytochrome C gave a single sharp zone and seems to be an excellent demonstration substance (see photograph; one cell contained cytochrome C and another a mixture of cytochrome C and human hemoglobin).

c) The proteins cannot be preserved with formaldehyde. Even at 0.1% formaldehyde, polymerization and insolubility effects were observed. An all purpose antibacterial agent (0.01 % gentamycin) served well over a one week period of storage at 37°C.

ATTEMPT TO GROW HUMAN CELLS IN PLASTIC CUPS FOR APOLLO 16 PROGRAM

Experiment 1 - Viability of cell cultures at 37°C for 18 hours.

Protocol

Three human cell lines and a mouse cell line were tested on Lexan plastic.

1. Cells were placed in holes drilled in the Lexan after the plastic plate was sterilized in the autoclave.

2. Human cell lines, (1) Hela, (2) RPMI 8226, (3) RPMI 1788 and (4) mouse L-929 cells were placed in the holes with culture medium. The cells were incubated at 37°C for 18 hours and then counted for viability and total cell count. (RPMI 1788 and 8226 cells grow in suspension.)

Results

1. Hela, L-929 and RPMI 8226 decreased about 50% in viability in 18 hours at 37°C. RPMI 1788 cells decreased about 18%.

2. Total viability/ml decreased in the cells containing Hela, L-929 and RPMI 8226. RPMI 1788 did not decrease in total viable cells/ml.

The decrease in total cell count may have been associated with the cells sticking to the plastic rather than remaining in suspension.

Experiment 2 - Viability of Cells at 23°C for 3 days.

Protocol

1. Human cells, Hela, RPMI 8226, RPMI 1788 and mouse L-929 cells test in siliconized and non-siliconized plastic bars.

2. Lexan plastic, Lexan treated with silicone, Plexiglass and Plexiglass siliconized bars were inoculated with the four cell cultures.

3. The cells in the plastic containers were incubated at 23°C for 3 days.

Results

1. There appeared to be no great loss in viability by trypan blue count with Hela, RPMI 1788 and L-929 cells. RPMI 8826 demonstrated a loss of viability but the cells were only 56% viable in the precount before the test.

2. Hela and L-929 cells showed a loss in total viable count/ml which was believed due to cells sticking to the plastic. Siliconizing the holes did not appear to help keep the cells in suspension.

3. RPMI 1788 cells (a suspension cell) did not decrease in total viable cell count/ml.

4. RPMI 8226 (a suspension cell) had a small decrease in total viable cells/ml in the test.

Experiment 3 - Viability of cells cultures at 23°C for 6 days.

Protocol

1. Hela, L-929, RPMI 1788 and RPMI 8226 were inoculated into holes in the following plastic containers - (1) Lexan; (2) Lexan-treated with silicone; (3) Plexiglass; (4) Plexiglass treated with silicone.

2. The cells were inoculated into the holes in the plastic bars and incubated for 6 days at 23°C.

Results

1. RPMI 1788 seems to show little loss of viability during the 6 day incubation period. RPMI 1788 had approximately the same number of viable cells/ml after 6 days as the initial inoculum.

2. L-929 cells also did not decrease in viability during the 6 day period, however, there was a sharp decrease in viable cells/ml.

3. Both Hela and RPMI 8226 cells showed a decrease in viability over the 6 day test period. Hela cells appeared to stick to the side and bottom of the wells.

APPENDIX II

ELECTROPHORESIS OF POLYSTYRENE LATEX

EXPLANATION OF TERMS AND ABBREVIATIONS USED IN THE FOLLOWING TABLES

SAMPLE: PSL 1p .109μ - 1p .357μ - 4p 5% Sucrose/.0015M Buffer

This indicates: Polystyrene Latex 1 part .109μ particle size plus 1 part .357μ particle size plus 4 parts Buffer solution (Buffer solution is listed under "Buffer") and its molarity plus 5% Sucrose on a weight to weight basis to Buffer.

BUFFER: $NaLSO_4$ indicates sodium lauryl sulfate, measured on a weight to weight percentage basis to Buffer.

Formalin was measured on a volume to volume percentage to Buffer.

GRADIENT: Percentages indicate amount of Sucrose, on a weight to weight basis, to Buffer.

VOLT: Indicates applied potential and if preceeded with a negative sign indicates a reversal of potential.

CURRENT: First figure indicates current at start of run. Second figure indicates current at end of run.

ELECTRODES: Pt = Platinum, H+/Pd = Electrolized palladium

pH At End of Run: A = Anode C = Cathode

REMARKS AND RESULTS: Stringing at forward edge - indicates a falling away of some of the sample forming tentacle like streamers coming from forward edge of sample.

Definition describes clarity of leading and trailing edges of sample

Separation (mm) indicates distance between trailing edge of faster moving sample and leading edge of slower moving sample.

The term sample band was used when no separation was evident and indicates the compactness of sample.

SINGLE COOLED CELL

SAMPLE	BUFFER	GRADIENT	CELL WALL TREATMENT	DATE	Volt	CURRENT	Time Min.	Elect-rodes	Tmp of Cool-ant	pH at end of run	REMARKS	RESULTS
PSL 1p .357μ-1p .79μ-4p 5% sucrose/.0015M Buffer	TRIS-HCl pH 8.5 .0015M	5% - 20%	Collodion Coated	9-1-71	300	.62 → .48 ma	30	Pt	ICE H_2O	—		No separation, definition-Fair - Sample band 1".
" "	"	"	"	9-1-71	300 -300	.80 → .84 ma .84 → .98 ma	13 20	Pt	"	—	After release no power applied for 30 min.	After 30 min. sample had settled only 1 1/4" from origin although some stringing was noted at forward edge. No separation.
PSL 1p .357μ- 1p .79μ-3p 5% sucrose/.0015M Buffer	"	"	"	9-1-71	300	.74 → .22 ma	60	H+/ Pd	"	—	Some flocculation	Possible start of separation. Definition - Fair.
PSL 1p .109μ- 1p .79μ-3p 5% sucrose/.0015 M Buffer	"	"	"	9-1-71	300	.76 → .28 ma	60	"	"	At 40 Min. C-8.8	"	Definite separation (17 mm) Definition - Fair.
PSL 1p .109μ- 1p .79μ-4p 5% sucrose/Buffer	(H_3BO_3-NaOH) (.0016M) Borate pH 8.5 + .1% $NaLSO_4$	"	"	9-9-71	300	.64 → .44 ma	40	"	"	—	System cooled in refrig. before use.	Separation (19 mm). Definition - Good.
PSL 1p .109μ- 1p .79μ-4p 5% sucrose/Buffer	Borate pH 8.5 w/0 $NaLSO_4$	"	"	9-9-71	300	.40 → .26 ma	35	"	"	—		Possible separation. Forward edge good definition.
PSL 1p .109μ- 1p .79μ - 4p 5% sucrose/Buffer	Borate w/.05% $NaLSO_4$	"	"	9-10-71	300	.90 → .28 ma	40	"	"	A-7.7 C-8.9		Separation (15 mm) Definition - Good.
PSL 1p .109μ- 1p .79μ 4p 5% sucrose/Buffer	Borate w/.05 $NaLSO_4$ + .1% - Formalin	"	"	9-16-71	300	.92 → .53 ma	40	"	"	A-6.7 C-8.7		Separation (19 mm) Definition - Fair-Good
" "	Borate w/0.1 $NaLSO_4$ + .1% - Formalin	"	"	9-17-71	300	.96 → .48 ma	40	"	"	A-7.0 C-9.1		Separation (20 mm) but very hazy between bands.

SINGLE COOLED CELL (page 2)

SAMPLE	BUFFER	Gradient	Cell Wall Treatment	DATE	Volt	CURRENT	Time mia.	Electrodes	Tmp of coolant	pH of end of run	REMARKS	RESULTS
PSL 1p .109μ- 1p .79μ- 4p 5% sucrose/Buffer	Borate w/.1% $NaLS0_4$ + .1% Formalin	5% - 20%	Collodion Coated	9-17-71	300	.91 → .45 ma	40	H+/ Pd	ICE H_20	A-7.2 C-8.9		Separation (21 mm) Definition-Good
" " "	" "	"	"	9-20-71	150	.56 → .28 ma	60	"	"	A-7.2 C-8.8		Separation (12 mm) Poor definition - haze between bands.
PSL 1p .234μ- 1p .79μ- 4p 5% sucrose/Buffer	" "	"	"	9-23-71	300	13.6 → 5.4 ma	40	"	"	A-6.9 C-8.9		Separation, but almost looks like 3 bands
" " "	" "	"	"	9-23-71	300	11.2 → 7.8 ma	35	"	"	A-6.8	Newer tube Fresher coating	Separation (10 mm) Definition not too good
" " "	" "	"	"	9-23-71	215	.9 → .88 ma	30	"	"	A-7.3 C-8.8	Membrane broke Sample diluted	Separation (7 mm) Definition Fair-Good
" " "	" "	"	"	9-24-71	210	.8 → .46 ma	55	"	"	A-7.3 C-8.8		Separation (10 mm) Definition-Fair
PSL 1p .109μ- 1p .79μ- 4p 5% sucrose/Buffer	" "	"	"	10-1-71	300	1.18 → .24 ma	40	"	"	A-7.2	Fresh stock buffer	Separation (22 mm) Definition-Very Good
PSL 1p .234μ- 1p .79μ- 4p 5% sucrose/Buffer	" "	"	"	10-1-71	300	1.12 → .64 ma	35	"	"	A-6.3 C-8.6		Separation (27 mm) Definition-Good
" " "	" "	"	"	10-4-71	300	1.16 → .20 ma	60	"	"	—	Newly coated tube baked 2 hr. 100°F Stand 4 day Dist. H_20	Separation (20 mm) Definition-Fair Haze between bands
" " "	" " pH 9.1	"	"	10-4-71	300	1.50 → .50 ma	40	"	"	A-7.8 C-9.3		Definite separation, but extremely smeared & diffused
PSL 1p .234μ- 1p .79μ- 4p 5% sucrose/Buffer	$NaHC0_3$ .003M pH 8.0 .1% Formalin .05% $NaLS0_4$	"	"	10-5-71	300	1.46 → .32 ma	50	"	"	A-6.8 C-9.9	Anode part of cell 8.0 Cathode p/o/c 9.5	Separation (10 mm) Definition-Fair
" " "	" "	"	Silicone Grease	10-5-71	300	1.37 → .38 ma	50	"	"	A-6.9 C-10.1	Anode part of cell 8.1 Cathode p/o/c 9.2	Separation (22 mm) Definition-Good
PSL 1p .234μ- 1p .79μ - 4p 5% sucrose/Buffer	Borate pH 8.5 (.0016M) + .1% $NaLS0_4$ + .1% Formalin	"	"	10-6-71	300	1.10 → .32 ma	35	"	"	A-7.0 C-8.6	Anode part of cell 6.4 Cathode p/o/c 7.3	Separation (20 mm) Definition-Good
No Sample	" "	"	Collodion	10-7-71	300	2.80 → .79 ma	30	"	"	—	Measured temp. on outside center of tube	

SINGLE COOLED CELL (page 3)

SAMPLE	BUFFER	Gradient	Cell Wall Treatment	DATE	Volt	CURRENT	Time min.	Elec-tro-des	Tmp of cool ant	pH at end of run	REMARKS	RESULTS
PSL 1p .109 μ- 1p .79μ - 4p 5% sucrose/Buffer	Borate pH 8.5 (.0016M) + .1% $NaLS0_4$ + .1% Formalin pH (8.2)	5% - 20%	Collodion	10-22-71	300	.90 → .68 ma	30	Pt	ICE H_20	A-7.7 C-7.7	Measured temp. on outside center of tube 8°C & circulated Buffer 8cc/min.	No separation - sample smeared over 1 1/2"
" " "	" "	"	"	10-25-71	300	.90 → .60 ma	40	Pt	"	A-7.4 C-7.4	Measured temp. on outside center of tube 6°C & circulated Buffer 8cc/min.	Separation - Poor Definition - Poor
" " "	" "	"	"	10-25-71	300	1.65 → 1.22 ma	25	Pt	40°C	A-7.5 C-7.5	Same except 40°C	Separation - Poor Definition - Poor
PSL 1p .234μ- 1p .79μ 4p 5% sucrose/Buffer	" "	"	"	10-26-71	300	1.90 → 1.25 ma	20	Pt	40°C	A-7.5 C-7.5	Same except 36°C	Separation (21 mm) Definition-Good (Some haze between bands)
" " "	" " (sterile)	"	" (sterile)	12-20-71	300	1.65 → 1.15 ma	22	Pt	TAP H_20	-	Sterilization of cell in Et0/F-12 Solutions by autoclave	Separation (13 mm) Definition-Good

FLIGHT PROTOTYPE

SAMPLE	BUFFER	GRADIENT (sucrose)	Cell Wall Treatment	DATE	V	CURRENT	Time min.	Electrodes	pH at end of run	REMARKS	RESULTS
PSL 1p .357μ - 2p 5% sucrose/.0015 Buffer	TRIS-HCl pH 8.5 .0015M	5% - 20%	1 cell used (A-14 cell) None	7-26-71	300V	.7 ma → .3 ma	-	Pt	-	Density of gradient high. Sample rose when released.	Somewhat diffused band ~ 1" long.
Same as above	Same, except .0030M	Buffer - 20%	"	7-28-71	-		-	"	-	Aborted leak at anode.	
Same as above	"	"	"	7-29-71	-		-	"	-	Aborted. Membrane at anode ripped.	
Same as above	"	"	"	7-29-71	-		-	"	-	Tube from gradient maker broke.	Experiment aborted.
PSL 1p .357μ - 2p 5% sucrose/.0030M Buffer	"	"	2 cells used A-14. 1 cell Collodion coated.	7-29-71	300	.6 ma → .3 ma	60	"	-	Collodion dried 24 hours.	Collodion side badly smeared. Uncoated side - good definition ~ 1/2" long.
Same as above	"	"	"	7-30-71	-		-	"	-	Collodion dried 24 hours then baked 100°F for 1 hour.	Aborted due to leak.
Same as above	"	"	"	7-30-71	-		-	"	-	Aborted due to failure in gradient maker.	
Same as above	"	"	"	8-2-71	300	.3 ma	120	"	-	Poor continuity in Collodion coated cell.	Collodion coated: 1" long. Front streamers. Uncoated: Excellent definition. 1/4" long
PSL 1p .109μ - 1p .357μ - 4p 5% sucrose/Buffer .0015M	"	"	"	8-2-71	300	1.4 → .7 ma	60	"	-	Uncoated tube moved 3/16" further than coated tube.	Separation in both cells. Good definition. Separation ~ 4 mm in both.

SAMPLE	BUFFER	GRADIENT (sucrose)	CELL WALL TREATMENT	DATE	V	CURRENT	Time Min	Elect-rodes	pH at end of run	REMARKS	RESULTS
PSL 1p .109μ- 1p .357μ- 4p 5% sucrose in Buffer .0015M 4p 5% sucrose in Dist. H_2O	TRIS-HCl pH 8.5 .0015	Buffer - 20%	2 cells used A-14 1 cell Collodion coated	8-3-71	300V	.7 →.5ma	45	Pt	-	Mylar strip broke. Had to be perforated	Coated tube slower than uncoated. Coated tube more diffused sample.
Same	Same	Same	Same	8-3-71	300V	1.3 →6 ma	40	Pt		Poor continuity in collodion coated tube for first 10 min.	Coated tube - poor definition, slower rate. Uncoated tube - good definition.
PSL 1p .109μ- 1p .357μ- 8p 5% sucrose/Buffer .0015M	Same, except .0030M	Same	Same	8-3-71	300V	4.0 →.8 ma	30	Pt		Uncoated cell moved ~ 7/16" faster.	Uncoated cell - good definition band ~ 9/16" - coated cell excellent definition - band at 7/16"
Same	Same, except .0060M	Same	Same	8-3-71	300V	6.0 → 1.0 ma	30	Pt	A-8.3 C-9.1	Uncoated side slightly (1/4") faster than coated cell	Very slight separation in both
Same	Same, except .0030M	Same	Same	8-4-71	300V	2.0 →.7 ma	35	Pt		Poor continuity in 1 cell, leak in other	No valid results
PSL 1p .109μ- 1p .357μ 8p 5% sucrose/Buffer	Fisher (SOB-139) Cert. Buffer pH 9.0	Same	Collodion coated cell, coated over w/gelatin	8-4-71	300V	7.0 → 9.0 ma	16	Pt	A-11.5 C-8.1	Uncoated-moved faster, both very smeared over half length of cells. Coated cell sample become floccular.	
Same	Same	Same	Same	8-5-71	225V	2.5 ma	-	Pt		Mistake in sucrose conc.	Aborted
Same	Same	Same	Same	8-5-71	225V	5.0 ma	25	Pt		Sample def. and size were about the same. Coated cell moved 1" further in 25 min. (Cont. not checked.)	No separation

SAMPLE	BUFFER	GRADIENT (sucrose)	CELL WALL TREATMENT	DATE	V	CURRENT	Time Min.	Elec-trodes	pH at end of run	REMARKS	RESULTS
PSL 1p .109μ- 1p .357μ 8p 5% sucrose/Buffer	Same, except Dil. 2:1	Same	Same	8-6-71	300V	3.0 ⟶ 3.5 ma	20	Pt		At end of 20 min. no separation. Coated cell moved faster than uncoated. Power off 15 min. Some sep. noted in coated cell sample.	
Same, except .0015M	TRIS-HCl pH 8.5 .0030M	Same	Same	8-6-71	300V	3.5 ⟶ .6 ma	45	Pt		Possible very slight sep. Both samples moved same rate.	Definition excellent in both.
Same	Same	5% - 10%	Same	8-9-71	300V	3.0 ⟶ 1.2 ma	33	Pt		Rate - ~ same. No separation. Gradient was too dense. Samples moved up before starting down.	
PSL 1p .109μ- 1p .357μ 8p 7.5% sucrose/.0030M Buffer	Same	5 % - 10%	Same	8-9-71	300V	5.0 ⟶ 2.0 ma	15	Pt		Sample too dense.	
PSL 1p .109μ - 1p .357μ 8p 6.3% sucrose/.0030M Buffer	Same	Same	Same	8-9-71	250V	4.0 ⟶ .7 ma	55	Pt		Both same rate. Samples about 1" long. Migrating faster down sides of tubes.	Poor definition

SINGLE UNCOOLED CELL

SAMPLE	BUFFER	Gradient	Cell Wall Treatment	DATE	V	CURRENT	Time Min	Electrodes	pH at end of run	REMARKS	RESULTS
PSL 1p .109μ - 1p .357μ 8p 5% sucrose/.0030M Buffer	TRIS-HCl pH 8.5 .0030M	5% - 10%	None	8-11-71	300V	1.4 → 1.4 ma	10	Pt	-		Sample ~ 1" long with poor definition - no separation.
PSL 1p .109μ - 1p .357μ 4p 5% sucrose/.0015M Buffer	"	Buffer-20%	None	8-20-71	225V	1.5 → 1.5 ma	25	Pt	-	After 15 min. sample ~ 3/4" - after 25 min. long floccular tail.	Fair definition. No separation.
" "	"	"	None	8-20-71	300	1.5 → 1.3 ma	15	Pt	-	No separation	Leading edge good definition. Trailing edge poor - band 1" long.
" "	Same, except .0015M	"	None	8-20-71	200	.9 → 1.0 ma	6	Pt	-	Sample moved length of tube in 6 min., 1 1/4" band.	Poor definition, sample appeared slightly too dense.
Same, except 4p Buffer .0015M	"	"	None	8-20-71	210	.9 → .88 ma	25	Pt	-	Front face good definition. Trailing edge - fair, no separation - sample ~ 7/8" long.	- - -
PSL 1p .79μ - 2p 5% sucrose/.0015M Buffer	"	"	Collodion Coated	8-27-71	300	1.1 → 1.2 ma	35	Pt	A-7.9 C-9.1	Anode solution had been refreshed 4 X during run.	Sample was diffused and contained floccular material.
PSL 1p .357μ - 1p .79μ 4p 5% sucrose/.0015M Buffer	"	"	"	8-27-71	300	1.1 → .94 ma	25	Pt	-	Very floccular and diffused	No separation
" "	"	"	"	8-27-71	300	1.1 → 1.0 ma	20	Pt	-	Used another cell.	Same as above
PSL 1p .357μ - 1p .79μ 6p 5% sucrose/.0015M Buffer	"	5% - 20%	"	8-31-71	300	1.28 → .64 ma	25	Pt	-	Diffused, floccular	No separation
PSL 1p .357μ - 3p 5% sucrose/.0015M Buffer	"	"	"	8-31-71	-	-	-	-	-	It was noted that after release sample was moving with any applied volt. It was found that dialysis membrane was leaking.	
" "	"	"	"	8-31-71	300	1.3 → 1.1 ma	15	Pt	-	Dialysis membrane put on dry instead of wet and doubled.	Sample band 1 1/2" long. Good definition on leading edge.

OTHER EXPERIMENTS

Evaluation of a Salt Bridge 8-30-71

A salt bridge was constructed by infiltrating a porous polyethylene plug in a glass tube, with polyacrylamide gel (7.5 g PA + 150 cc .0015 M TRIS-HCl buffer pH 8.5 + .3 cc DMAP + 300 mg APS).

The tube containing the gelled plug was attached to a plastic bottle (cathode reservoir) by means of a rubber stopper. The bottle had had the bottom removed and was used in an inverted position. To the other end of the glass tube a dialysis membrane was attached by means of an "O" ring. This end was suspended in a beaker (anode reservoir). Platinum electrodes were used for both electrodes.

Concentrated TRIS-HCl (.015 M) pH 8.5 buffer was placed in cathode reservoir + .0015 M TRIS-HCl pH 8.5 buffer in the snode reservoir and tube.

Current changes were monitored at an applied potential of 300 V.

Over a 25 minute period current rose from 4.0 ma to 9.0 ma. pH at end of run - Anode: 3.5, Tube: 3.8, Cathode: 8.5.

Testing Migration Rate of Chloride Ions through Salt Bridge 9-3-71

Salt bridges were formed, as described in Evaluation of a Salt Bridge, 8-3-71, in each end of a glass tube which also contained a side tube. An inverted polyethylene bottle with a cutaway bottom was again used as a cathode reservoir and a beaker as an anode reservoir. Electrolyte in cell (between salt bridge) was .278M $NaHCO_3$ buffer, pH 9.0 plus enough conc. NH_4OH and $AgNO_3$ to just keep silver from precipitating. Final pH was 9.3.

Anode and cathode reservoirs were filed with 1M KCl. Electrodes were both Ag-AgCl.

Filled cell allowed to stand 5 minutes without applying potential. No signs of any chloride leakage through salt bridges.

Potential across cell ranged from 3.5 volts at start to 2.0 volts at end of 105 minutes. Current from 2.2 ma to 2.0 ma. At the end of 50 minutes a slight precipitate started to form below cathode salt bridge.

Testing Migration Rate of Chloride Ion Through Salt Bridge 9-22-71

Same set up and reagents as used in Test Migration Rate of Chloride Ion Through Salt Bridge, 7-3-71, except porous plug held in place with RTV rubber.

Cell filled and allowed to stand 10 minutes with no potential applied. No signs of chloride leakage.

Potential across cell ranged from 1.6 volts at start to 1.0 volts at 270 minutes. Current remained constant at 2.0 ma. At no time was any precipitate observed.

Testing Effects of .1% Sodium Lauryl Sulfate ($NaLSO_4$) on the Gore-Tex Membrane of the Phase Separator 9-13-71

An Apollo 14 phase separator was filled with borate buffer .0016M pH 8.5 containing .1% $NaLSO_4$. A reservoir was attached one foot above separator so as to place a constant 1 ft. head on the system. System was observed for 14 days. No apparent signs of wetting were noted but 40-50 cc of solution were missing from reservoir. Some water vapor was present on inside of plastic jacket.

Testing Gore-Tex Membrane in Apollo 16 Phase Separator 5-3-72

Using a peristaltic pump, borate buffer, .0016M, pH 8.5 with 0.1% $NaLSO_4$ + .1% formalin was pumped through an Apollo 16 phase separator at about 8 cc/minute for 50 days. During this period cavity in separator block filled to about 3/4 full (about 10 cc) and remained this way. No apparent areas of wetting were noted in Gore-Tex membrane surface.

Testing Shelf Life of Borate Buffer and Additives 11-1-71

Samples stored in closed polyethylene bottles for 26 days. Starting pH 8.3 in all samples.

Results after 26 days:

1. Borate Buffer: pH 8.0
2. Borate Buffer + .1% $NaLSO_4$ w/v: pH 8.2
3. Borate Buffer + .1% formalin v/v: pH 7.8
4. Borate Buffer + .1% $NaLSO_4$ w/v + .1% formalin v/v: pH 8.1.

Testing Palladium-Electrolized Palladium Electrode System 8-24-71

Buffer: Borate pH 9.0
Anode: Electrolized palladium ($H^{\pm}/_{Pd}$)
Cathode: Palladium
Applied Volt: 300
Current: ~ 7.0 ma
Gas Evolution: Anode-None, Cathode-None
pH at end of 180 minutes test - Anode 9.4 Cathode 8.5

Effects on Palladium-Electrolized Palladium Electrode System, When Immersed in Buffer System Overnight

Buffer: Borate pH 9.0
Anode: Electrolized Palladium ($H^{+}/_{Pd}$)
Cathode: Palladium
Immersion Time: 18 hours
Applied Volt: 300 V
Current: Rose from 8.5 ma ⟶ 30 ma
Test Time: 60 minutes
Gas Evolution: None at either electrode
Buffer removed and replaced with fresh Buffer
Applied Volt: 300
Current: 7.0 ⟶ 2.5 ma
Test Time: 35 minutes
Gas Evolution: None

Testing Effectiveness of Electrolized Palladium Electrodes in Combatting pH Changes of Buffer Solutions at Anode and Cathode Reservoirs 9-16-71

A single cell was set up as normal, both with reservoirs containing 30 cc of borate buffer pH 8.5 .0016M with .05% sodium lauryl sulfate w/w and .1% formalin v/v. Tube was also filled with buffer solution.

Electrodes - both electrolized Palladium ($H^+/_{Pd}$) 1 cm x 2.5 cm x .005" thick.

Current was maintained at .80 ma for 60 minutes (applied potential increased from 122v to 340v). During this period pH at anode dropped from 8.6 to 8.12 and at cathode rose from 8.6 to 8.77.

At end of 60 minutes current was lowered to .40 ma and maintained there due to limitations of power supply. Applied potential over the next 60 minutes increased from 170 to 180 volts. During this period pH at anode continued to fall from 8.12 to 7.60 and at cathode rose from 8.77 to 8.90.

Testing Effectiveness of Electrolized Palladium Electrodes in Combating pH Changes of Buffer Solutions at Anode and Cathode Reservoirs 8-22-71

Repeat of above experiment except borate buffer contained KCl (instead of 50 cc 0.1 M H_3BO_3 + 10.1 cc 0.1 M NaOH diluted to 600 cc distilled H_2O, 50 cc of a mixture 0.1 M with respect to both KCl and H_3BO_3 + 10.1 cc 0.1 M NaOH diluted to 600 cc distilled H_2O was used).

Current was maintained at 1.5 ma for 120 minutes (applied potential increased from 47 to 187 volts). During this period pH at anode fell from 8.25 to 3.0 and pH at cathode rose from 8.25 to 9.1.

From the tube at about where the forward band of latex would be pH was 7.75 at end of 120 minutes.

Effects of Electrolized Palladium Electrodes on Buffer Solution 9-15-71

3 Plastic bottles were filled with borate buffer pH 8.6 containing .05% $NaLSO_4$ w/v + .1 % formalin v/v.

A Palladium electrode was suspended in one bottle while an electrolized Palladium electrode was suspended in another. One bottle was as a control.

At the end of 34 days the pH of each bottle was tested:

1) Control - 8.1
2) Pd - 7.8
3) H^+/Pd - 5.3

Measuring Temperature Changes of Electrophoretic Cell 10-6-71

Buffer: Borate pH 8.5 w/.1% $NaLS0_4$ + .1% formalin
Electrodes: Electrolized palladium (both)
Thermocouple: (Copper-Constantan) taped to tube at mid-point

Applied Potential: 300 Vdc
Run Time: 30 minutes
Current: 2.80 ma .79 ma
Temperature: Rose from 72°F to 81°F in first 10 minutes then dropped to 76.5°F over next 20 minutes
Applied Potential Reversed - 300VDC
Run Time: 30 minutes
Current: .79ma 2.50 ma
Temperature: Gradually rose from 76.5°F to 93°F

Testing Effects of Circulating Buffer Between Anode and Cathode Reservoirs — 10-12-71

Buffer: borate buffer pH 8.4 with .1% sodium lauryl sulfate w/w + .1% formalin v/v.

Dialysis membrane at both ends of cell.
No cooling.
Buffer circulation 8cc/min.
Electrodes: Platinum
Applied Potential: 300VDC
Run Time: 40 minutes
Current: 2.8 ma → .86 ma
pH End of Run: Anode 8.3 Cathode 8.3
Reversed Polarity
Applied Potential - 300 VDC
Run Time: 40 minutes
Current: .82 ma → 4.3 ma
pH End of Run: Anode 8.15 Cathode 8.0

pH of Buffer in Tube at End of Test
Anode End: 8.2
Cathode End: 8.15

Testing Temperatures of Apollo 16 Pump and Pumped Liquid — 10-12-71

Pumped Liquid: H_2O (~ 8cc/min.)

Pumping Time - 80 minutes
Temperature: Pump - $73^{\circ}F \rightarrow 118^{\circ}F$ H_2O - $71^{\circ}F \rightarrow 80^{\circ}F$
Pump was resting on a stainless steel bench top

Repeat of above except a 1" wide braided aluminum strap was wedged between heat sink plates and taped to stainless steel bench top.
Temperature at end of 80 minutes: Pump: $75^{\circ}F \rightarrow 113^{\circ}F$
H_2O: $72^{\circ}F \rightarrow 78^{\circ}F$

Testing Effects of Circulating Buffer Between Anode and Cathode Reservoirs

10-14-71

Parameters Measured: Cell Temperature (wall at mid-point), pH changes, current, applied potential: potential across cell (middle 50 mm, 1/2 length of cell).

Buffer: borate .0016M pH 8.4 with .1% sodium lauryl sulfate w/w + .1% formalin v/v.

Dialysis Membrane at both ends of cell.

No cooling or temperature control.

Buffer circulation 8cc/min.

Electrodes: Both platinum.

Applied Potential: 300 VDC

Run Time: 30 minutes

Potential Across 50 mm of Tube (1/2 length): 126v ⟶ 36v

Current: 3.2 ma ⟶ .78 ma

Temperature: $70^0F \xrightarrow{10\ min.} 83^oF \xrightarrow{20\ min.} 76^oF$

pH at End of Run: Anode 8.2 Cathode 8.15

Reversed Polarity after 30 minute wait

Applied Potential: -300 VDC

Run Time: 30 minutes

Potential across 50 mm of tube (1/2 length) -21v⟶ -122v

Current: .70 ma ⟶ 3.48 ma

Temp: $72^oF \xrightarrow{20\ min.} 89^oF \xrightarrow{10\ min.} 72^oF$

pH at end of run: Anode 8.1 Cathode 8.0

pH in cell: Anode end 8.3, cathode end 8.4

APPENDIX III

ASTRONAUT OPERATING AND TRAINING MANUAL FOR THE APOLLO 16 FLUID ELECTROPHORESIS DEMONSTRATION

INTRODUCTION AND MEANING OF THE DEMONSTRATION

Electrophoresis, meaning "borne by electricity" is a process used widely in clinical testing and in medical research. It is one of the mildest and most effective ways of separating complex, delicate biological materials. But while electrophoresis is almost indispensable to the clinical analyst, it has not proven very useful as a means of producing pure biologicals in significant amounts. This is largely due to problems with convection and sedimentation (both due to gravity) in large scale operations.

By doing electrophoresis in future orbiting space vehicles where there is essentially zero-gravity, we hope to produce some of the world's purest biologicals and vaccines. We also hope to separate useful quantities of living cells - living normal cells from diseased cells, thymus-dependent lymphocytes from bone-dependent lymphocytes - separations of inestimable value to the researchers who are trying to find cures or vaccines for cancer, leukemia, or other such diseases.

On Apollo 16 we are trying to prove that in the absence of gravity it is possible to electrophorese large particles in a manner impossible on earth. The demonstration unit contains three tubes filled with fluid. Into each tube will be injected some polystyrene latex (small plastic beads) which are a fairly good simulation of some of the cells we hope one day to separate. (Real cells were not possible on Apollo because of their difficult storage requirements.) When voltage is applied to the sample tubes, the polystyrene latex will move slowly from left to right. One of the three tubes contains a mixture of two polystyrene latexes, representative of two kinds of cells. In this tube we should be able to see separation of the sample into two distinct bands by the time the sample has moved to the right-hand end of the tube. The other two tubes serve as sort of checks on the separation tube.

PROCEDURE

A. INITIAL SET-UP

1. Remove demonstration unit (Figure 1) from storage location and place it in the operating location.

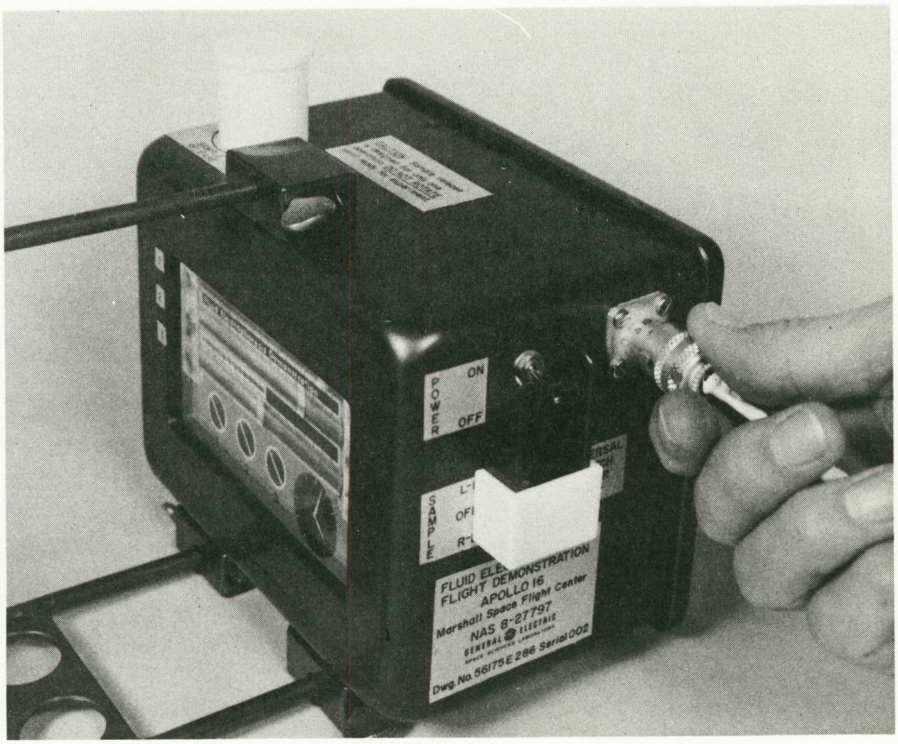


Figure 1

2. Attach mounting straps or springs to hold the unit firmly in place, window side facing out.

3. Prepare 70 mm Hasselblad camera for photographing the demonstration.

   a. Attach 80 mm lens to the extension tube on tripod that was stowed with the electrophoresis box. (Figure 2).

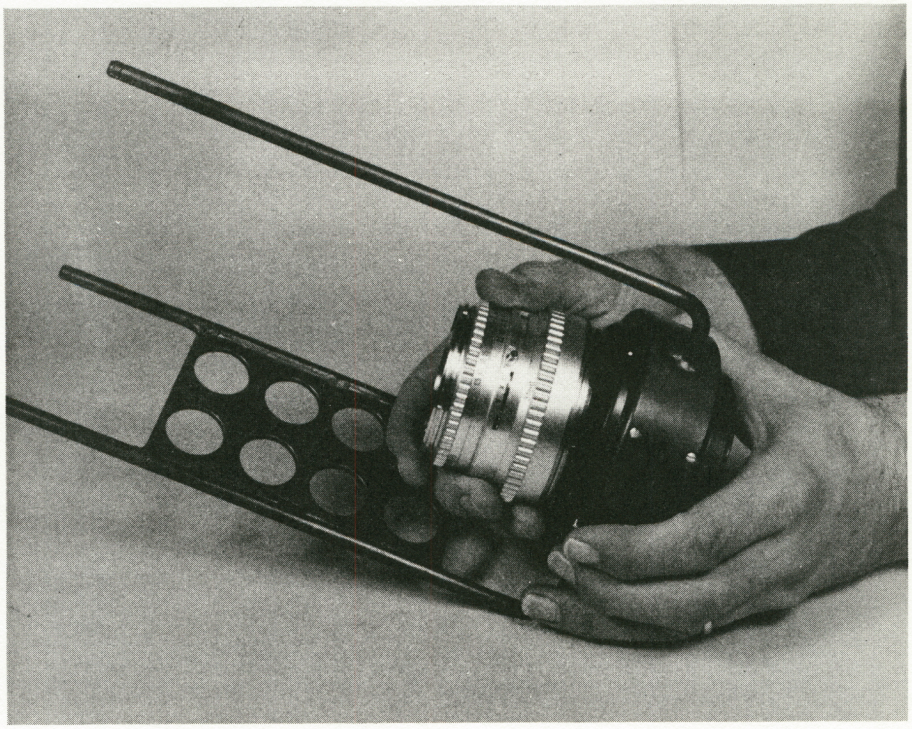


Figure 2

b. Attach film back to the Hasselblad camera body.

c. Mount camera body on the extension tube plus tripod system.

d. Mount tripod with camera on the electrophoresis box. (Figure 3).

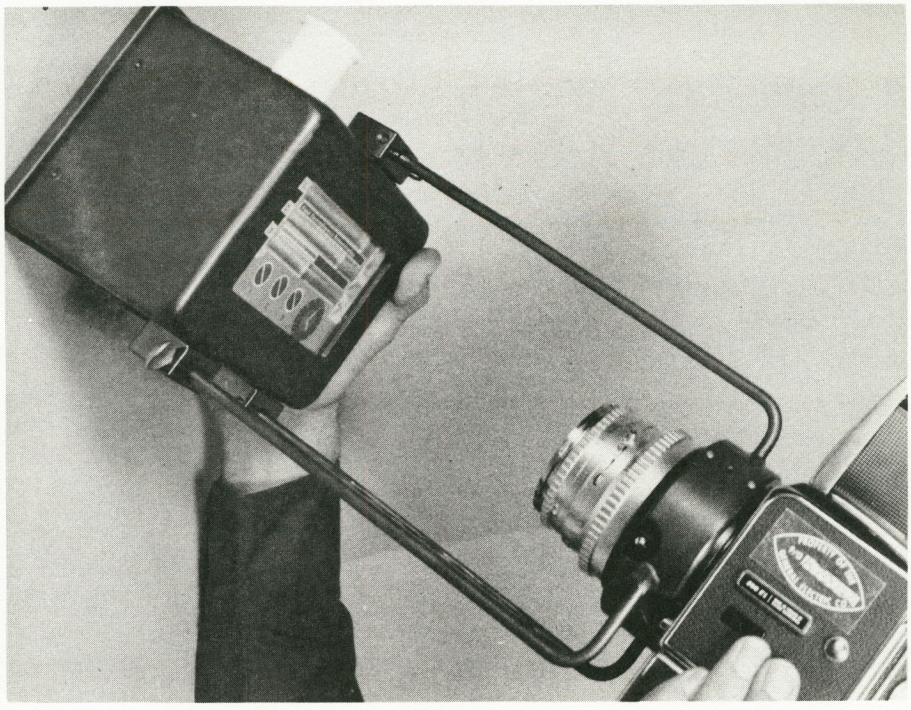


Figure 3

e. Tighten thumbscrews at base of tripod.

f. Make proper camera settings of aperture, range and exposure time.

(Set aperture to
(Set range to feet
(Set exposure time to second.

g. Make mechanical and electrical connections to the intervalometer.

4. a. Be sure power switch is off and sample release knob-cover is in place. Allow the unit to remain motionless for about 10-15 minutes so that any sloshing or mixing of fluids settles down.

b. Verify PNL 201 power - off.

5. Connect 115V, 400 Hz (A.C.) (vacuum cleaner) power cord to electrophoresis demonstration unit and to spacecraft outlet (PNL-201). (Figure 4).

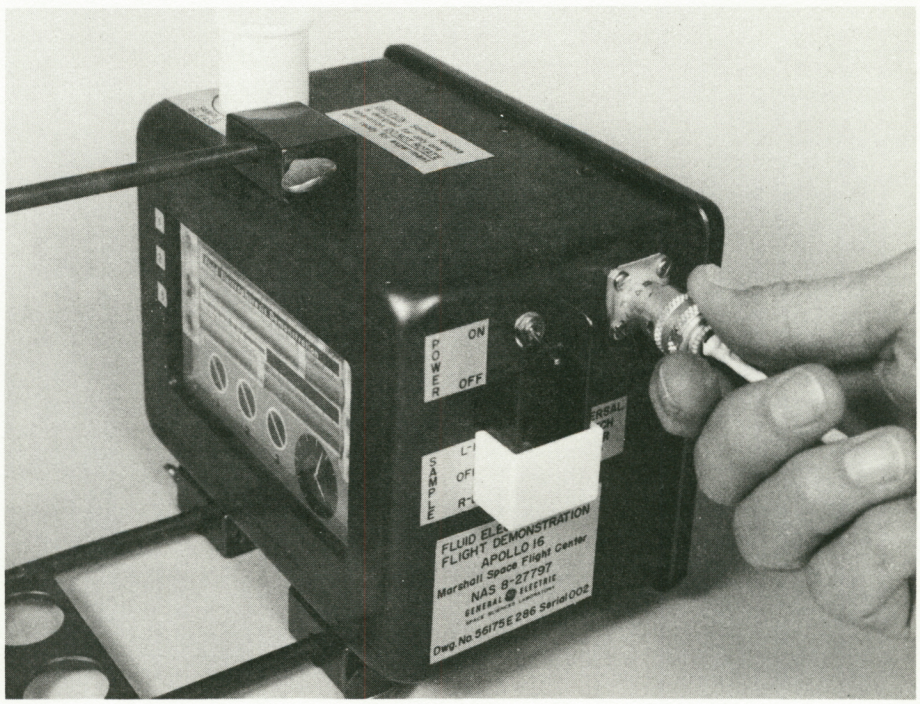


Figure 4.

6. Dim lighting in spacecraft to low level suitable for safe movement around the cabin.

7. Place shades over spacecraft windows as required to prevent sun shafting on the unit during photography.

B. DEMONSTRATION START-UP

(After 10-15 minutes period mentioned previously in 4.a.:

1. Turn on power switch which activates pump, motor, cell voltage and lights (Figure 5).

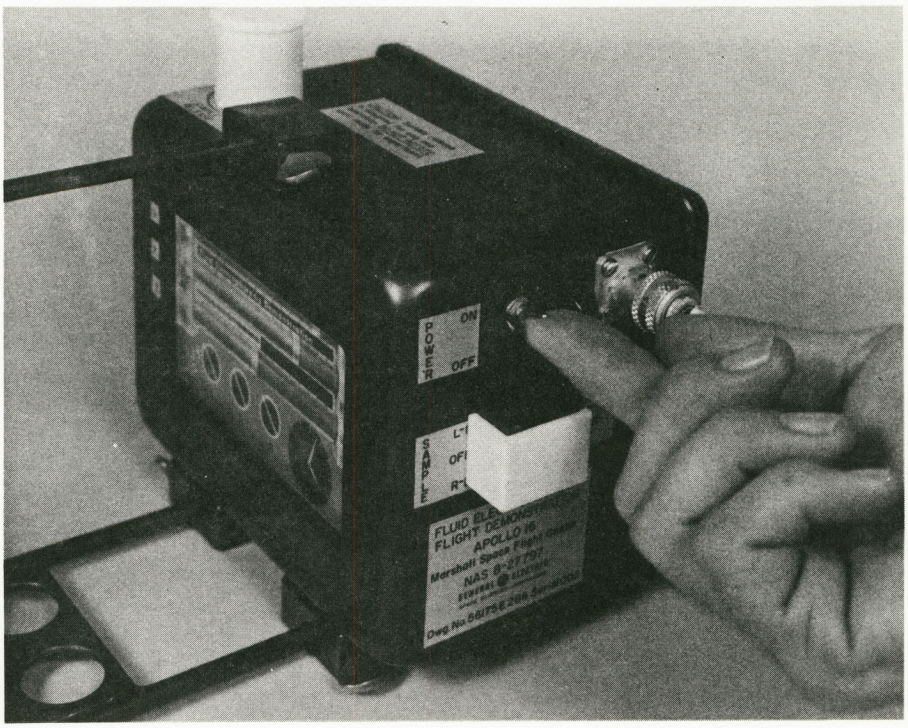


Figure 5

2. Observe the orange Kapton film which contains the polystyrene latex at the far left of the cell block. This film will be removed during Step B.4.

3. Remove cap from the sample release knob and discard.

4. Rotate sample release knob slowly and steadily one-half turn in direction of arrow to start demonstration. This removes the Kapton film from around the sample and the sample will begin to move. (Figure 6).

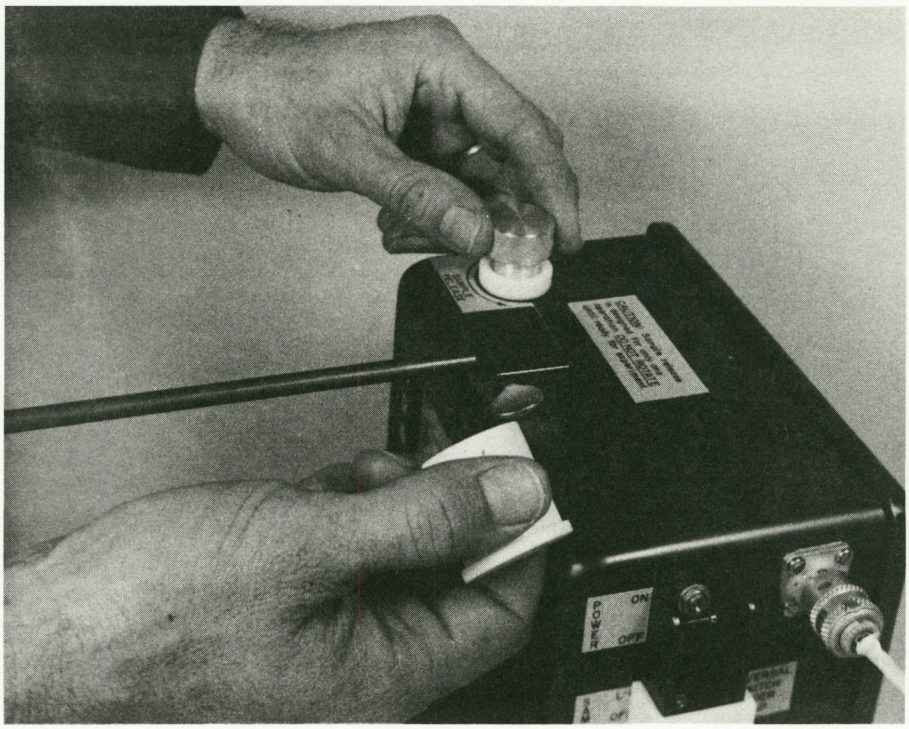
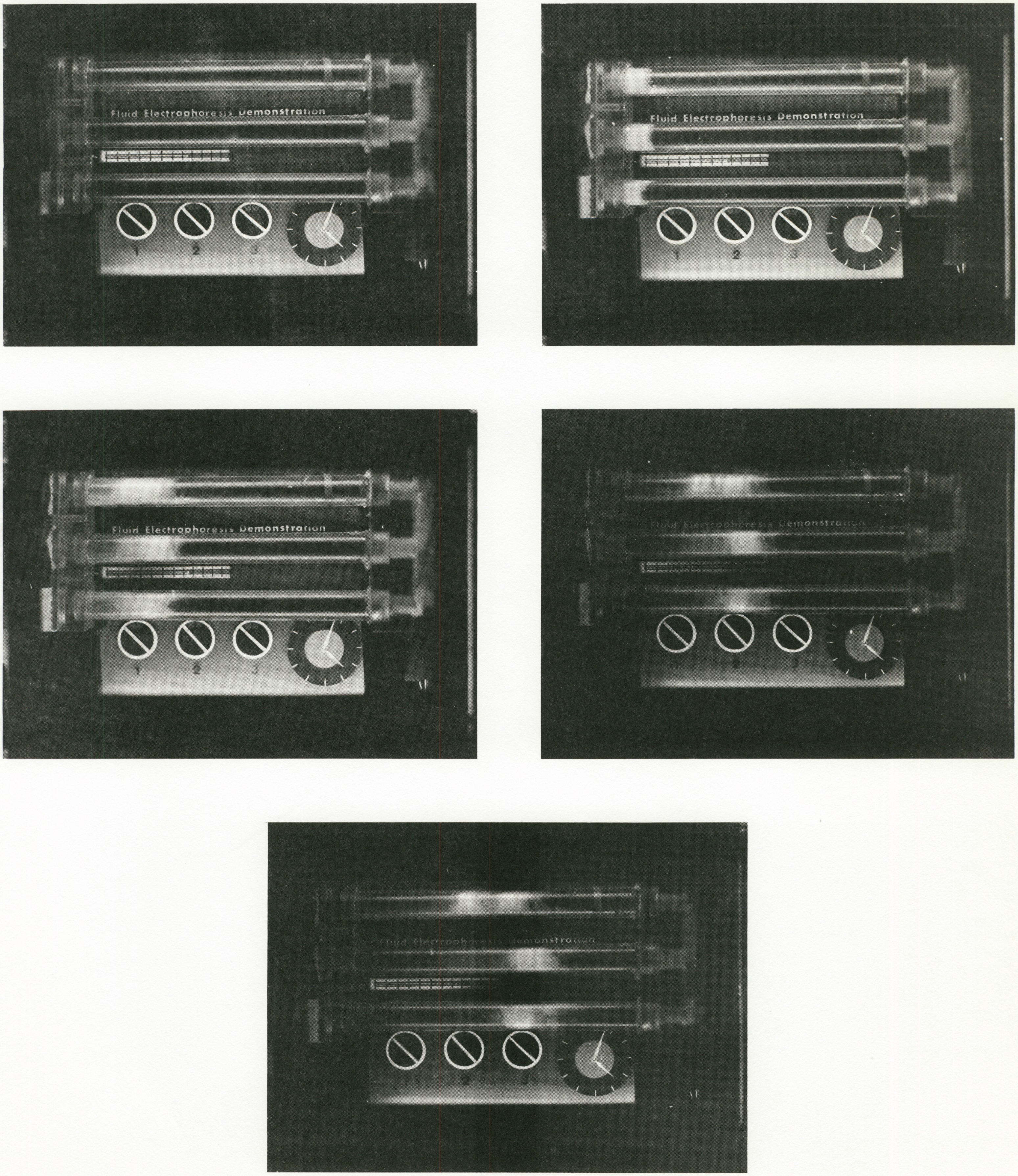


Figure 6

5. Take first pictures as soon as possible after completion of the previous step. The intervalometer will now automatically sequence the subsequent pictures in 20-second intervals.

6. During the next twenty-five minutes, the three groups of particles will drift across the tubes and be photographed automatically.

   A series of pictures taken at about six minute intervals shows approximately how the experiment should look:



8A

7. When the leading sample approaches the yellow band in the upper tube, remove and discard the cover from the sample reversal switch.

8. Move this sample reversal switch to the "R - L" position while continuing automatic photographic sequence. (Figure 7).

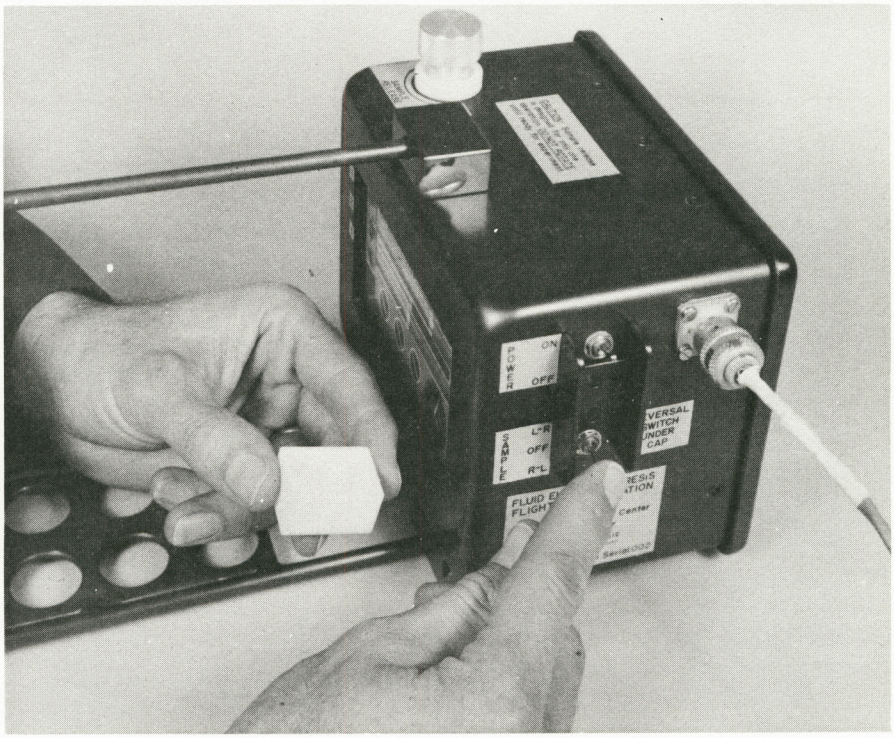


Figure 7

9. Complete photographic sequence utilizing 160 frames of the 70 mm film.

10. The demonstration is now complete.

    a. Turn off the power switch

    b. Remove the camera and tripod

    c. Remove and stow the exposed film cassette

    d. Remove mounting straps and return the electrophoresis box to its stowage container.

VI. CONTINGENCY INSTRUCTIONS

A. FAILURE OF POWER SUPPLY TO CELL ELECTRODES

1. Same power-on switch supplies electrodes and motor. If, after power is turned on, the motor fails, continue operating instructions, but be prepared to observe no flow of particles.

2. If no flow is observed, check power switches, power receptacles, and spacecraft circuit breaker. If all of these are okay and no flow is observed, experiment has failed.

B. SAMPLE RELEASE KNOB

If the sample release knob does not remove the Kapton film around the particles, no particles will enter the demonstration tubes. This can be confirmed by observing orange Kapton film still surrounding specimens.

C. BURSTING OF DIALYSIS MEMBRANES

If a membrane between the specimen solution in the tube and the electrode cap bursts, the specimen solution will flow through the electrolyte recirculation system. No harm is done, but that tube demonstration has failed.

D. LEAK OF ELECTROLYTE CIRCULATION SYSTEM

A leak in the electrolyte circulation system is not harmful but may slowly deplete electrolyte from the electrodes and stop the demonstration.

VII. CALIBRATION

The electrophoresis demonstration unit requires no calibration.

VIII. MAINTENANCE

No maintenance is described as the electrophoresis demonstration unit is repairable only at the manufacturer's facility.

APPENDIX IV

CONTAMINATION CONTROL AND STERILIZATION PLAN

FOR APOLLO ELECTROPHORESIS EXPERIMENT EQUIPMENT

1 August 1971

Martin G. Koesterer
Microbiologist
Life Systems Operation

TABLE OF CONTENTS

SECTION I

INTRODUCTION

One of the prime objectives of the Space Processing and Preparation of Materials Project is to determine the feasibility of performing separation and purifying new and unique high-quality, high-value materials such as pharmaceuticals and biologicals. To carry out such experiments successfully, it has been necessary to establish a plan for microbial contamination and control and sterilization that insures hardware which is free of undesirable contaminating micro-organisms. The overall approach planned to accomplish the objective is to sterilize the hardware and experimental materials separately by the most compatible means and then aseptically add the materials to the hardware system.

This document has been prepared as a general project plan to outline the various procedures or activities required from the time of early design to meet the contamination-free constraints.

It is expected that the plan will be revised and updated as required and implemented on the design and fabrication of the proposed hardware for the Apollo 16 flight during the last quarter of 1971.

SECTION II

BACKGROUND

1.0 BACKGROUND

While it is acknowledged that no formal policy of quarantine or sterilization specifically applies to the experiments planned for the Apollo series of space-flights, contaminated experimental hardware could lead to influencing the out-come of the experiment. Experience gained from the experiments flown on earlier missions indicated that microbial contamination can and will occur to high levels unless necessary precautions are taken to avoid it during manu-facture assembly and test. Hence, microbial contamination is to be avoided. Thus, it is essential to render the hardware microbially free and prevent the hardware from becoming recontaminated for not only duration of the experiment, but for the duration of the specific mission through post-flight operations.

2.0 OBJECTIVES

The Contamination Control Plan will have the following objectives for preparation performing and analyzing the results of the particular electro-phoresis experiment.

2.1 All aspects of the proposed experiment including assembly, test, and post-flight analysis of the flight hardware, shall be examined in order to identify the conceivable sources of microbial contamination.

2.2 Each separate source of contamination will be investigated to yield an adequate understanding of the process through which it occurs, so that adequate means of controlling it can be determined.

3.0 SOURCES AND MODES OF CONTAMINATION

The following outline of all currently anticipated sources and/or modes of contamination is presented to define more carefully the nature of the modes/ and the sources which constitute each mode, and to illustrate the problem areas which must be investigated or controlled to fulfill compliance with the objective:

3.1 Contamination of Experimental Hardware as it is being manufactured and assembled.

- o Handling - human
- o Environment

3.2 Contamination of the experimental materials

- o Experimental chemicals or reagents
- o Buffer filling solution.

3.3 Failure of the Sterilization Procedure

3.4 Failure to maintain identity during aseptic assembly

3.5 Recontamination of the assembled hardware system containing the experimental materials after aseptic assembly; i.e., during test, performance, post-flight analysis.

SECTION III

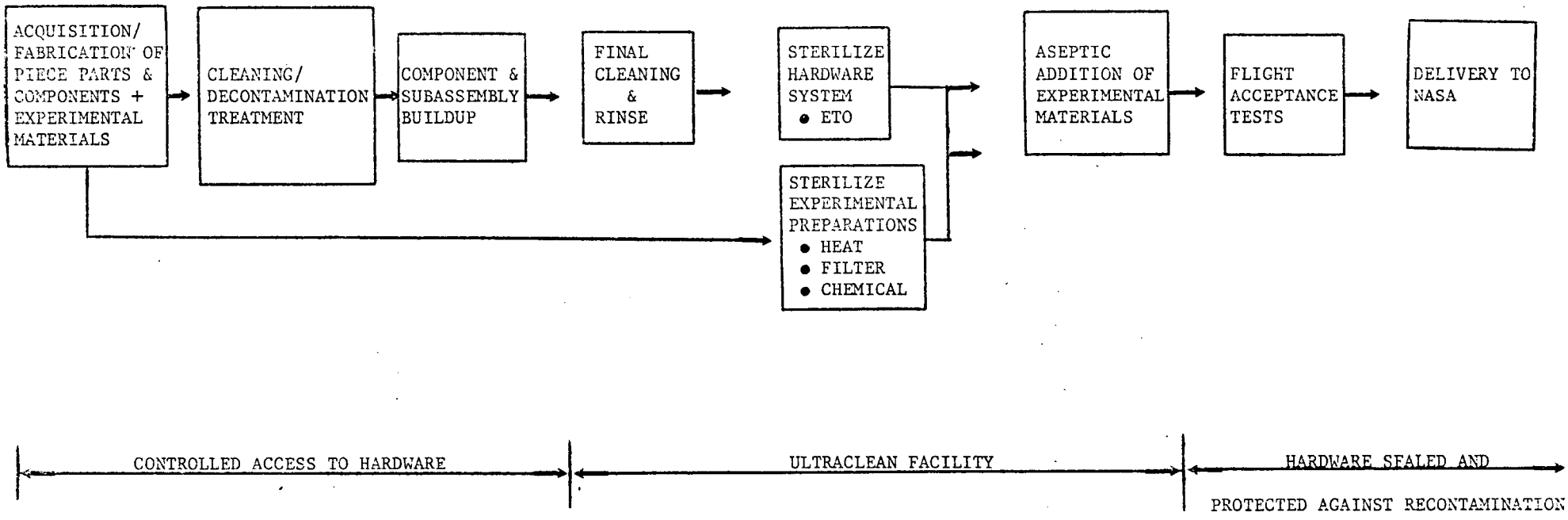
APPROACH

The proposed approach would utilize a separate sterilization of the hardware system utilizing a "cold" sterilization technique and an appropriate technique compatible to the experimental materials, bring the two together aseptically in a sterile atmosphere. The proposed approach utilizes proven techniques which can be implemented with minimum perturbations on the existant hardware, schedule and have only a minor increase in the manpower required.

The following is a general description of the approach.

The hardware will be sterilized by ethylene oxide in the germ-free isolator. The materials will probably be sterilized by filtration (final method dependent upon specific materials selected for experiment (Section IV-2)) in the clean bench. The sterile materials will be added to the hardware in the clean room and the hardware sealed and protected by appropriate packaging.

FIGURE 1. ASSEMBLY FLOW AND STERILIZATION OF ELECTROPHORESIS FLIGHT HARDWARE



SECTION IV

STERILIZATION

1.0 HARDWARE STERILIZATION

1.1 Introduction

The following approach and procedure have been elaborated to sterilize the Electrophoresis hardware with gaseous ethylene oxide in a flexible isolator. The diagram depicts the concept and equipment involved. The detailed procedure for sterilizing the Electrophoresis hardware with ethylene oxide follows:

1.2 Scope

This document defines the operating procedures for sterilizing the Apollo Electrophoresis system (cell, electrolyte flow system, and associated components) with ethylene oxide. It pertains to the performance of a) treatment in a flexible isolator system and is assumed to be compatible with the acknowledged materials of construction[1] and b) retrieval of an adequate representative sample of rinse fluid on which analysis for presence of any microorganisms will be performed. The specific test procedures are defined as precisely as possible within the limitations of the equipment employed and have been designed to have minimal impact on the assembly procedure.

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[1]This assumption is based on generally available information on the compatibility of ethylene oxide and the materials identified as comprising the hardware. No special testing was performed and no liability is accepted by the operating component.

1.3 Purpose

The sterilization procedure is designed to render space flight hardware (or engineering test models) free from viable microorganisms by destroying any such organisms remaining in the system after manufacture, assembly and test. It is intended that sterile solutions of the experimental materials will be admitted to the hardware aseptically and the system be capable of storage for a period of months without recontamination. The reason is to avoid microbiological deterioration of the hardware system, experimental materials until the experiment is completed during the planned Apollo 16 flight, currently scheduled for March, 1972.

1.4 Principle

The hardware to be sterilized is placed in a chamber made of plastic film equipped with glove ports, microbially retentative filters, and a means for admitting the sterilizing agent. The sterilizing agent (ethylene oxide diluted with dichlorodifuluoromethane ("Freon" 12), a non-flammable mixture in air) is admitted to the deflated chamber and expands and inflates it. A means of mechanically insuring circulation of the sterilizing atmosphere throughout the system to be treated will be provided (vacuum or pressure pump, and fan) as required. The sterilizer system is maintained in thos mode for an adequate period of time and then the sterilizing mixture exhausted by air dilution for a period of time sufficient for the desorption of any ethylene oxide which may have dissolved in such organic materials as rubber (gloves, "0" rings) or plastics, paints and the like.

It is then possible to perform filling and sampling operations aseptically in the sterile environment or in an adjacent bioclean environment, and when completed remove the sealed hardware in a protective package for delivery to NASA for installation aboard the Apollo spacecraft.

1.5 Apparatus

The sterilization apparatus consists of a plastic film exposure chamber and ethylene oxide-dichlorodifluoromethane mixture packaged in liquified state in metal containers under pressure. Drawings and specifications are available from G-F Supply Division, Standard Safety Equipment Company (Palatine, Illinois), and Pennsylvania Engineering Company, Incorporated (Philadelphia, Pennsylvania), respectively.

1.5.1 A. Chamber

The basic chamber is approximately 24 inches wide by 24 inches high by 36 inches long (approximately 12 cubic feet volume) and of 12 mil polyvinyl chloride film. The base of the isolator is mounted to a mobile table or bench. While being used as a sterilizer, it is kept inflated by the gaseous sterilizing mixture and at other times by sterile filtered air. The chamber has several openings and they are fitted with several accessories: the largest 12 inches in diameter, provides access for admitting and removing hardware to be sterilized; three other 1-2 inch diameter tubular openings are provided for admitting the sterilizing gas mixture, for adding air, and for exhausting. Each is equipped with a microbial retentative sterile filter. The chamber was designed as a dual purpose unit in that it can be used not only to sterilize items with ethylene

oxide, but it also can be used as a chamber in which the hardware can be filled under sterile conditions, if required. Rubber gloves are attached to provide for aseptic manipulation of the hardware in the sterilized chamber.

B. Sterilizing Mixture

The chamber is sized to use approximately 2 to 2.5 pounds of pressurized gaseous mixture composed of 12 percent ethylene oxide and 88 percent dichlorodifluoromethane by weight, contained in a 140-pound cylinder (Pennsylvania Engineering Company, Philadelphia, Pennsylvania). This amount of mixture will inflate the isolator and achieve a concentration of approximately 450 mg of ethylene oxide per cubic foot in the chamber which should accomplish sterilization in a six to twelve hour exposure period at room temperature.

C. Exposure

Exposure will commence after: (a) the placement of the hardware and any associated equipment in the chamber; (b) plugging of all tubular connections to filters with rubber stoppers and employment of inner cap over the entrance port; (c) admission of moisture by means of an atomizer (DeVilbliss Nebulizer) or live steam from some steam generating source; or other means and (d) admission of the gaseous sterilizing mixture and attainment of inflation of the isolator from a collapsed condition.

DETAILED PROCEDURE FOR STERILIZING HARDWARE IN FLEXIBLE FILM ISOLATOR WITH ETHYLENE OXIDE

A. Preparation of Hardware/Equipment

1. Hardware should be as free of dirt and and organic matter as possible.
2. All connections and openings on the hardware which will allow penetration of the sterilizing gas should be loosened, opened, or freely exposed. The position of any valves should be positively established if possible.
3. A list of all materials, tools, etc., required in the isolator system to disconnect or connect the hardware is required and should be prepared, if aseptic filling is to be performed therein.
4. All materials required to rinse and fill the Electrophoresis system should be available and, if required, separately sterilized, prepared for placement in the isolator unit. (The actual filling and sampling may be performed in the bio-clean area.)

B. Preparation of Flexible Isolator and Associated Equipment

1. The interior surface of the isolator should be cleaned, washed and rinsed using warm soap and water.

2. All visible leaks and loose tube connections should be repaired.

3. The efficiency of the air filtration system should be checked for:

   (a) leaks in connections

   (b) pressure losses

   (c) conditions of filters

   (d) maintain a sterile back-up system (complete).

4. Some arrangement should be made to raise the item(s) to be treated off the floor of the isolator. Placing toweling on the floor of the isolator ensures that the under-side of objects in the cabinet will be contacted and thus sterilized by the ethylene oxide.

5. To avoid admittance of liquid ethylene oxide, the following means of vaporizing it completely should be included:

   (a) a coil placed in a pan or pail of hot water (60-80^{o}C) in the line between the ethylene oxide container and the chamber.

   (b) a trap-flask in the line from the dispenser into the chamber

   (c) a shallow pan containing toweling which will act as a baffle and absorbent inside the chamber.

6. A fresh supply of ethylene oxide sterilizing mixture, adequate in quantity to perform all treatments, should be on-hand and ready for use.

C. Admission and Placement of Equipment to be Sterilized to Treatment Chamber

1. Make a complete check of list of items that have to be placed in isolator during sterilization. Check these items from the list when placing them into the isolator.

2. Place all items in isolator on rack or toweling and separate from each other to allow good circulation of the sterilizing gas mixture.

3. Loosen or disconnect all fittings possible to allow good diffusion of the sterilizing gas mixture to all surfaces of the item to be sterilized.

4. Check to see that any materials such as water bottles (for sampling) and culture media are sealed so that the sterilizing gas won't penetrate them, if such operations are to be performed in the isolator.

5. When the isolator is loaded, seal the unit by slipping the cover back over the entrance opening and stretching the "O" ring over the cover. Plug the entrance and exhaust tubular opening from the chamber to the filters with the supplied rubber stoppers.

D. Humidification of Chamber

1. Admit the pre-determined amount of water vapor as live steam or atomized water (nebulizer) to raise the relative humidity to around 40-50% or greater.

2. Allow a 20-30 minute period for the humidity to equilibrate within the chamber. An accessory fan or blower can be used to aid in this.

E. Admission of the Gaseous Sterilizing Mixture

1. After the humidification equilibration time, the ethylene oxide - Freon 12 sterilizing mixture is admitted to the chamber through the gas conditioner (heat exchanger coil and trap) until the chamber is completely inflated. The admission of the gas may be through the tubing of the hardware to be sterilized or directly into the shallow pan and toweling baffle provided.

2. When the desired amount of sterilizing gas mixture has been added, the unit is left to complete the desired sterilizing exposure.

F. Exposure Period

1. Exposure timing will commence with the initial attainment of the fully inflated condition of the isolator with the sterilizing mixture and will extend for approximately 12-16 hours (overnight) to provide adequate time to accomplish the sterilization.

2. The internal fan or blower, if desired, should be operated during this period to assure complete exposure of the hardware and mixing of the sterilizing mixture components.

G. Removal of Sterilizing Agent from the Chamber

1. After completion of the specified exposure period, the plugs are removed from the chamber inlet and exhaust filters and the filter blower turned on to dilute and exhaust any unreacted sterilizing gas.

2. This exhaust procedure should be allowed to proceed for approximately 6 hours to allow complete desorption of any ethylene oxide by the rubber gloves on the isolator and the hardware materials.

H. Sterility Testing and Hardware Operations (Optional)

1. After removal of the residual and absorped sterilizing agent from the chamber, the appropriate samples or tests for sterility including culturing the microbial sterility indicators, if desired, should be taken or performed as soon as possible.

2. After all samples required for the appropriate sterility test have been taken, all manipulations, including removal to a bio-clean area may now be performed. Precautions should be taken to maintain sterility of the treated hardware by avoiding any handling or operations which would recontaminate it.

3. It may be desirable to hold the hardware in the sterile isolator for a day or two pending analysis of the sterility test.

2.0 EXPERIMENTAL MATERIALS

This section remains to be generated. It will be based upon the types and nature of materials yet to be decided upon for the experiment on Apollo 16.

SECTION V

ASEPTIC ASSEMBLY

1.0 APPROACH

The successful application of the approach (outlined in Section III) relies upon reliably bringing together the experimental materials and hardware aseptically in a sterile atmosphere. The techniques required to accomplish this are personnel trained in aseptic technique, use of sterile handling equipment and performance in a sterile or microbially free area. The personnel in the Life Systems Group have had much experience in these procedures and the utilization of the facilities depicted in the following sub-section for performing aseptic or sterile assembly.

2.0 FACILITIES

The facilities which are available for performing the aseptic addition of the experimental materials to the separately sterilized hardware consist actually of three somewhat different bio-barrier systems which will provide a sterile environment:

1) Performance of the operations in a "germ free" type isolator (Figure 1)
2) Performance of the operations in a Class 100 horizontal laminar Flow Clean Bench, shown by previous testing to provide a sterile environment (Figures 2, 3, and 4).
3) Performance of the operations in a Class 100 Laminar Down Flow Clean Room (possibly with a horizontal Laminar Flow Clean Bench) within the Clean Room) Figure 5.

All three of these type facilities are available in the Life Science Operation at the Valley Forge Space Center.

The selected approach will utilize all three types of facilities for one phase of the operation or another.

SECTION VI

STERILITY TESTING OF THE ELECTROPHORESIS HARDWARE

1.0 INTRODUCTION

The objectives of the sterility tests to be performed on the Electrophoresis system hardware and associated experimental materials are to determine by a culture test that the hardware is free of viable microorganisms after treatment with the selected sterilizing procedure, both immediately after treatment and after some months of storage after aseptic addition of the experimental materials.

The tests set forth in the following sections have been selected:

(a) To determine the presence of any residual viable organisms in the hardware or admitted during typical aseptic filling operations. This will be accomplished by assaying volumes of sterile rinse water flushed through the hardware system after the application of the sterilizing treatment.

(b) To determine that no viable microorganisms are added to the system by means of the experimental materials or method to be used to fill the container. This will be accomplished by microbiological assay of volumes of the materials prepared for the aseptic filling operation.

(c) To check on the efficiency of the sterilizing gaseous mixture (12% ethylene oxide; 88% freon; 12 by weight) under the conditions that the treatment is applied. This will be accomplished by exposing bacterial spore controls (filter paper strips containing high levels of viable bacterial spores known to be resistant to the chemical sterilant to be used) to the conditions in the chamber and the culturing of those controls upon completion of the treatment and to determine whether the test microorganism survived or was destroyed by that treatment.

3.0 STERILITY TESTING OF EXPERIMENTAL MATERIALS AS PREPARED FOR ADDITION TO THE ELECTROPHORESIS HARDWARE SYSTEM

3.1 Introduction

Basically, the sterility test consists of taking a representative sample of each of the experimental materials to be employed for filling the Electrophoresis unit and:

(a) Aseptically membrane filtering and culturing the contents as described below; or

(b) Placing representative aliquots (1 and 10 ml) into various sterile culture media as described below.

3.2 Assay for Aerobic Microorganisms

3.2.1 Membrane Filter Assay

Three aliquots of rinse water (~10 ml) shall be collected aseptically from each of the prepared supply of materials to be used for filling the experimental cells. If delivery can be made directly aseptically to sterile filtration apparatus, then three samples can be processed in that manner. If direct aseptic delivery is not possible, then three 10 ml aliquots will be collected in three respective sterile bottles provided for the purpose.

Ten ml of each of the materials shall be admitted to the funnel portion of a sterile millipore membrane Filtration Apparatus (Millipore Filter Corporation, Bedford, Maine). The water shall be passed through a 47 mm 0.22 micron pore diameter membrane filter by pulling a slight vacuum (5 inches of mercury) on the vacuum flask on which the membrane filter apparatus is supported.

The membrane filter is then aseptically transferred to either:

(a) Large diamter screw cap culture tubes or 40 ounce screw cap bottles containing sterile Trypticase Soy Broth; or

(b) To a Petri plate containing a poured layer of sterile Trypticase Soy Agar.

A shallow cover layer of sterile agar may then be poured to just cover the filter.

The tubes or plates containing the filters are incubated at 35°C for 7 days.

3.2.2 Sterility Test of 1 and 10 ml Aliquots of the Materials

As both a check on the techniques and materials used in the membrane filtration assay and as further indication of sterility, several (usually six) tubes of sterile Trypticase Soy Broth are inoculated respectively with 1 ml and 10 ml aliquots (three tubes each).

The inoculated tubes are incubated also at 35°C for 7 days and observed for any evidence of microbial growth (production of turbidity being the primary evidence).

3.3 Anaerobic Microorganisms

3.3.1 Sterility Test of 1 and 10 ml Aliquots of Water

An analysis for the presence of any viable anaerobic microorganisms will be conducted by aseptically transferring 1 and 10 ml aliquots of each of the materials into three tubes each of freshly prepared and sterilized thioglycollate broth with no shaking or mixing. These tubes will be incubated at 35°C for 7 days and examined for evidence of microbial growth (turbidity being the primary evidence of microbial growth).

4.0 STERILITY TESTING OF ELECTROPHORESIS EXPERIMENT SYSTEM

4.1 Introduction

The sterility testing of the Electrophoresis experiment system will be indirect in the sense that the system will be rinsed with sterile water and the water then tested for sterility. Water recovered by rinsing the system will be assayed for the presence of viable microorganisms in exactly the same manner as that for the experimental materials; i.e.,

4.1.1 Aerobic Microorganisms

4.1.1.1 By assaying three 100 ml aliquots of the rinse water according to the membrane filter assay technique as per 3.2.1.

4.1.1.2 By assaying 1 and 10 ml aliquots of the rinse water by placing directly into three tubes each of Trypticase Soy and Thioglycollate Broth as per 3.2.2

4.1.2 Anaerobic Microorganisms

4.1.2.1 By assaying 1 and 10 ml aliquots of the rinse water by inoculating into Thioglycollate Broth as per 3.3.1.

5.0 CONTROL TESTS ON ENVIRONMENT AND PERFORMANCE OF MICROBIOLOGICAL (STERILITY) TESTS

5.1 Introduction

In order to ensure the least possibility of false positive sterility test results on an item of the nature of the Electrophoresis Experiment System necessary to pay strict attention to aseptic techniques and operations. It is mandatory to conduct the tests in an environment which is relatively free of microorganisms such as that provided by the sterile isolator or by a laminar flow room or hood that has HEPA (High Efficiency Particulate Air) filtered air. All microbiological (sterility) testing to be performed on samples, including transfer, plating, filtration, etc., will be done in a Class 100 Laminar Flow Clean Bench.

5.2 Check on Class 100 Bench

Control petri plates (pre-poured with sterile Trypticase Soy Agar) will be exposed during all sampling operations as a real-time control. The exposed plates will be incubated at $35^{o}C$ for 7 days and observed for any evidence of microbial contaminants.

5.3 Sterility Check (Control) of Culture Media and Equipment

In order to assure that any evidence for the presence of viable microorganisms does not result from non-sterility of equipment or supplies control tests will be run on these items wherever possible. Culture media filtration apparatus, filters, forcepts, etc., will be freshly prepared and sterilized and held in protected areas; sample tubes and flasks being incubated with the samples being tested.

2

5.4 Tests to Establish Ability of Culture Media to Support Growth of Microorganisms

In order to assure that the culture tests employed were capable of detecting the presence of low numbers of viable microorganisms, if they are present in the rinse water and experimental materials which could contain toxic ions, residual cleaning compounds, etc., all the culture vessels (tubes or bottles) presenting no indication of growth will be innoculated with 1.0 ml of an aqueous suspension containing approximately 10 viable cells of a typical vegetative bacterium (Escherichia coli).

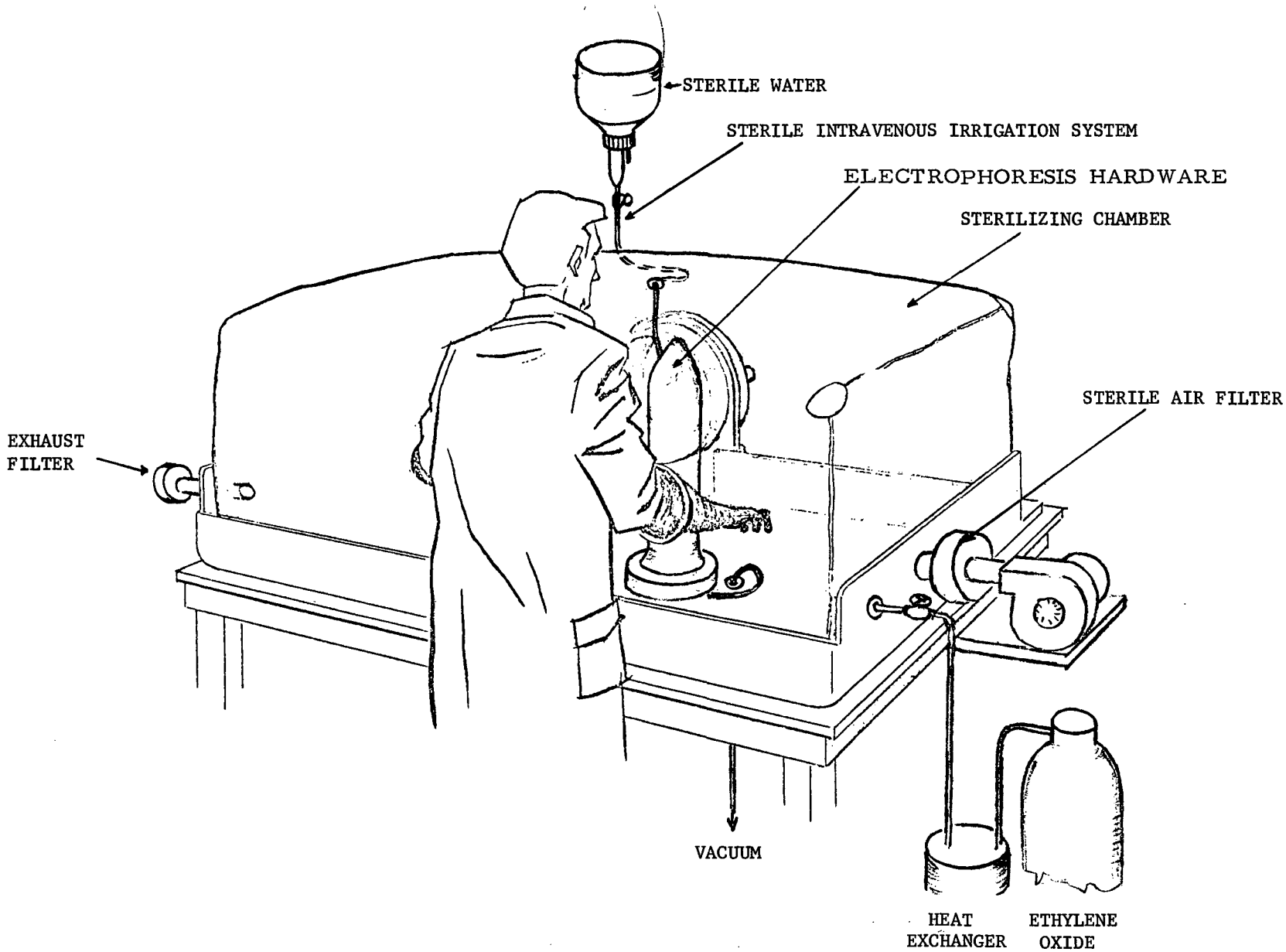

Figure 1. Sterilization and Aseptic Fill System for Electrophoresis Experiment in Germ Free Type Isolator System

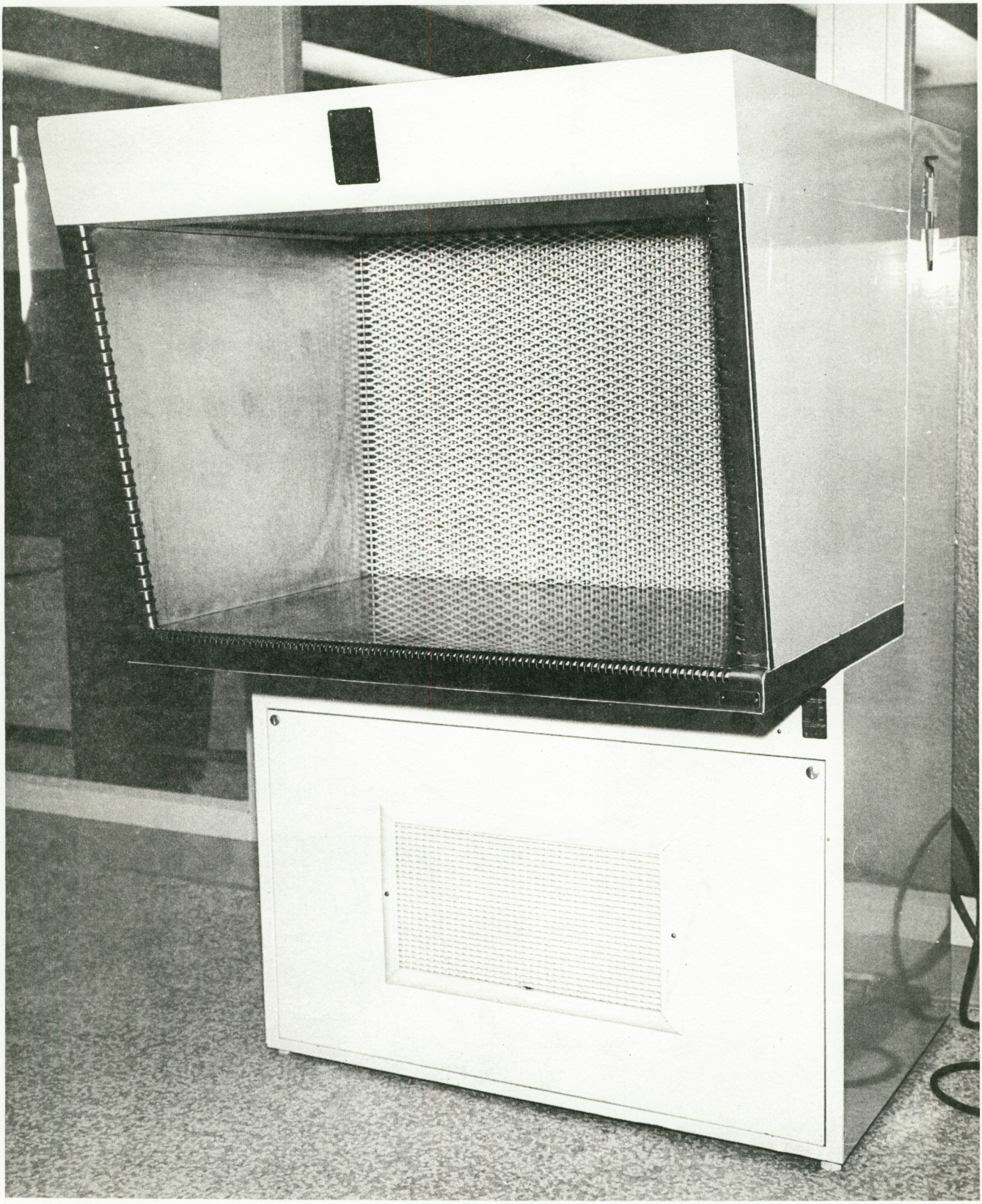

Figure 2. Class 100 Horizontal Laminar Flow Bench Used for Aseptic Microbiological Procedures

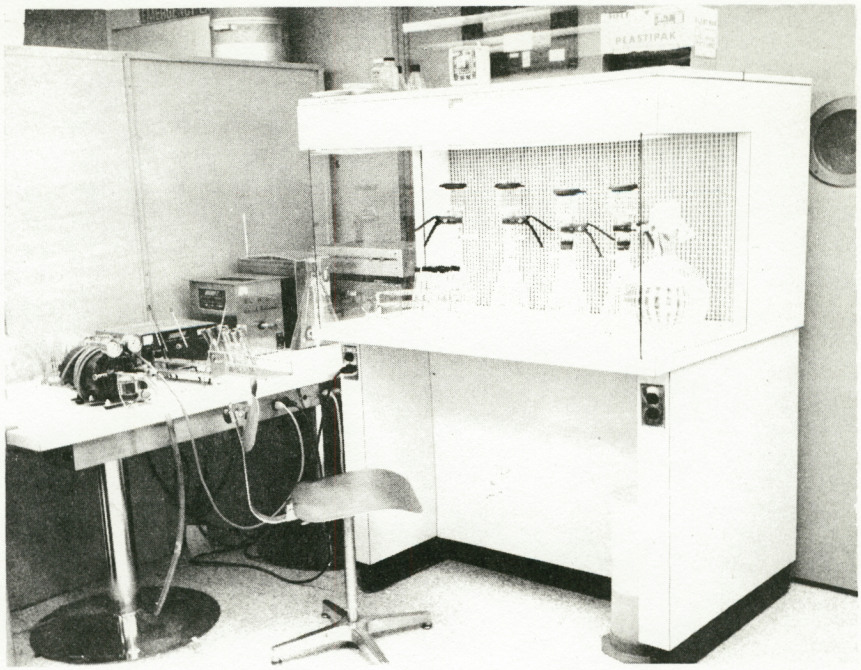

Figure 3. Typical Sterile Filtration Operation Set Up in Laminar Flow Bench

Figure 4. Typical Aseptic Microbiological Procedures Performed in Laminar Flow Bench

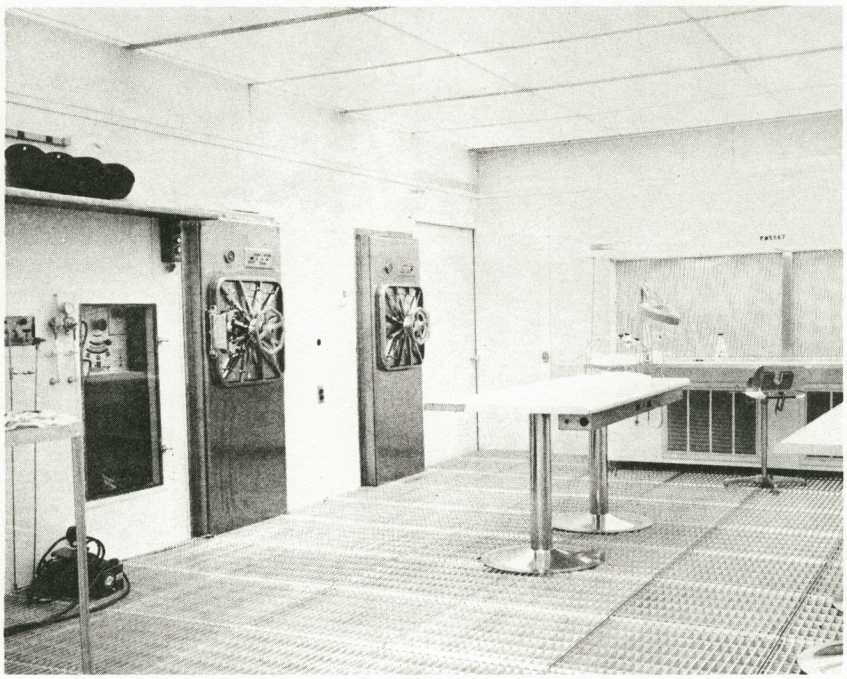

Figure 5. View of Life Systems Class 100 Laminar Downflow Clean Room For Performance of Aseptic Assembly Procedures

APPENDIX V

CRACKING FAILURES OF MACHINED LEXAN ELECTROPHORESIS CELL ASSEMBLIES PRODUCED FOR NASA (APOLLO # 16) BY G. E. CO. SPACE DIV.

D. W. CAIRD

PRESENT:

MARSHALL SPACE FLIGHT CENTER - NASA: Huntsville, Ala.

Dr. Robert S. Snyder, Contract Monitor - Materials Division

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Cecil Seaver, Q. C. Manager - Materials Division

GENERAL ELECTRIC COMPANY - SPACE DIVISION

Mr. Louis Mc Creight, Manager - Space Processing

Dr. Richard Griffin, Chemist

Dr. Joseph V. Mullin, Mechanics

Mr. Marty Koesterer, Microbiologist

Mr. Vic Mazzio, Chemist

GENERAL ELECTRIC COMPANY - SHEET PRODUCTS

D. W. Caird

1.0 BACKGROUND

An electrophoresis experiment for Apollo 16 is jeopardized by cracking failures of the Lexan cells. The author was first contacted by L. McCreight on March 9, 1972, and arrangements made to have cracked cell components (cell 004) forwarded to Pittsfield for examination and analysis together with a sample of buffer solution used in the cell. Shortly thereafter NASA requested an immediate meeting at Valley Forge to which

I was invited to help resolve probable environmental stress-cracking effects involved in the cracking failures.

2.0 MEETING SUMMARY

2.1 DISCUSSION OF PROBLEM

Both the prime and the back-up electrophoresis-cell assemblies for the Apollo 16 experiments have failed by cracking which has occurred both in (a) the cell header blocks and in; (b) the gas-phase separator covers. The cracking is not believed to have occurred during the first week following sterilization, assembly and filling with the aqueous electrophoresis buffer solution, and during which time the cells were tested for leakage etc. both at General Electric, Philadelphia and at MSFC (Huntsville). On subsequent storage, the cracking developed within a three-week period.

In cracked parts examined by the writer at the meeting, the cracking was found typical of environmental-stress origin.

2.1.1 STRESSES

Cracking was evident in different locations and to different extents, but in all cases it was associated with probable stresses; VIZ due to cemented-in inserts (both metal and Lexan), Lexan screw plugs, and metal screws. Also, machining stresses (machined parts have not been annealed) were also evident on birefrigence examination. Stress magnitudes are unknown at this time.

2.1.2 ENVIRONMENTS

I emphasized the important contribution of environmental contamination to stress cracking. Manufacturing, assembly and testing sequences were reviewed with this in mind. A number of possible or potential stress-cracking environments were identified to be evaluated and/or eliminated. These include:

(a) Machining Aids: An unidentified machining coolant and an aqueous detergent (unidentified) coolant have been used to date. In future production clean water alone will be adopted unless problems are encountered, in which case an "approved" coolant will be recommended by us.

(b) Cleaning of Machined Parts: Ultrasonic bath using reagent grade kerosene followed by petroleum either or N-Hexane rinse - air dried twenty four hour minimum (Note: at this time this procedure appears satisfactory).

(c) Sterilization Media: Freon 12/Ethylene Oxide (Mathieson)

(d) Adhesive Bonding: Epon 828/Versamide 140 adhesive cured 16 hour/RT plus 1 hour/150°F is used for bonding both Lexan and stainless steel, tubes into header blocks, etc. Possible effects on stress and compatibility are unknown.

Use of an RTV Silicone adhesive will be evaluated and adopted where possible.

(e) Miscellaneous Assembly Components:

Gasketing: - Vitron O-rings with Kel-F grease.

Kapton Film "Dam" Material

3.0 ACTION

3.1 General Electric Company is discontinuing use of the Freon-Ethylene oxide sterilization pending further compatibility testing. Parts will be machined using water only as a lubricant. Parts will be oven annealed @ 125° - 130° to relieve maching and forming stresses. RTV Silicone elastomeric adhesive will be used where feasible to relieve assembly strains

3.2 Examination of cracked parts and compatibility tests on components is being reported separately.

D. W. Caird:cas

3/27/72

cc:

J. Ballard

J. L. Cobb

L. McCreight

M. E. Priest

I. Examination of Electrophoresis cell returned from General Electric Company, Space Division, Philadelphia: Crack LOCI as received (refer photographs).

I. 1.0 TOP HEADER

1.1 Cracks # 1 and # 2 are circumferential cracks in the plane of the base of the tubing recesses of tubes #A and #B. (Note: No cracking present with tube C). Cracking initiates internally and at the sharp shoulder at the bottom of the recesses where the tubes with their cell membrane caps butt into the header. Crack origin areas are in immediate contact with the membrance material which is presumably saturated with buffer solution. External environments appear to be excluded except for possible retained contamination from machining, sterilizing or cleaning agents. Cracks do not extend to external surfaces.

1.1.1 Recommendation

Minimize present notch concentrated stresses by suitable RADIUS at the base of these recesses rather than the present sharp corners. It seems likely that membrane swelling (NEPHLEX MEMBRANCE-UNION CARBIDE CO.) may induce the stress condition in the observed cracking plane. If feasible use RTV silicone as a bonding sealant here.

1.2 Cracks # 6 and # 7 are RAIDAL CRACKS typical of hoop stresses originating at the tapped/threaded holes with Lexan screw plugs at the reat of the header. These cracks extend radially from the screw plugs associated with tubes A and B. They initiate at the external surface of the header block. They do not penetrate through to the tube recesses. Note Tube C plug is OK.

1.2.1 Recommendation

Minimize hoop stresses due to tightening the screw plugs by sealing with an RT Silicone adhesive rather than more imcompressible teflon tape now used.

1.3 Cracks # 8 and # 9 are RADIAL CRACKS originating at stainless steel tubes cemented into the header plenum. They are attributable to hoop stresses and initiate at or near the external surface.

1.3.1 Recommendation

Minimize hoop stresses attributable to tightness of fit and depend on adhesive for bonding and support. There is no evidence of cracking being induced by the Epoxy - ersmide # 140 adhesive used here.

2.0 BOTTOM HEADER

2.1 Cracks # 3, #4 and # 5 are radical cracks in the header block. These are typical of hoop stresses where tubes A, B and C are inserted and cemented into the r ecess holes in the bottom header. Cracking extends from the external surface and does not appear to penetrate through to the inner surfaces. There were no circumferential cracks as described in 1.1, because there tubes butt against a separate plate.

2.1.1 Recommendation

Minimize hoop stresses attributable to tightness of fit and depend on adhesive for bonding and support. Use elastomeric RTV silicone adhesive here if feasible.

2.2 Cracks # 10 and # 11 are radical cracks originating at the stainless steel tubes cemented into the plenum chambers of this bottom header. They are attributable to hoop stresses as discussed in P I.1.3 above (cracks # 8 and # 9).

2.2.1 Recommendation

As in P 1.3.1, plus if possible position the lower set of tubes as far away as possible from the threaded Lexan plugs in the base of this header.

II. Identification of High Stress Areas Via Carbon Tetrachloride Test.

1.0 Areas where cracks had already occurred as identified above were found to have mechanically imposed stresses associated with assembly which significantly exceeded 800 PSI (the critical stress level for CCl_4). Other identifiable high stress areas include:

1.1 Lamp brackets (supporting GE #F4TD-5 Lamps)

A screwed-on bracket cracked at the screw area. Also one of the lamps was held tighter than the other, probably due to alignment variation. The bending stress here caused cracking the brackets arms. Adhesive bonded joints of the other three brackets were all free from cracks.

1.2 Top Header Assembly

Cracking occurred at metal tube, Lexan plug and Lexan tube inserts. There was no cracking due to machining in areas not associated with mechanical assembly stress. There was no cracking due to bonding per se.

1.3 Bottom Header Assembly:

Occurences of cracking were similar to those for the Top Header as discussed above (P I 1.2).

1.4 Roller Bracket

One bracket cracked between screw-mount holes. The bonded-in bracket was uncracked.

1.5 Electrophoresis Cell Tubes

All three tubes (A, B, C) cracked; with cracks apparently initiating at the calibration rings machined into the surface. These tubes exhibited internal longitudinal tension apparently associated with their manufacture which would be relieved by pre-annealing particularly in all tubes where machinery' is employed as in this case.

1.6 Reservoir Tubes

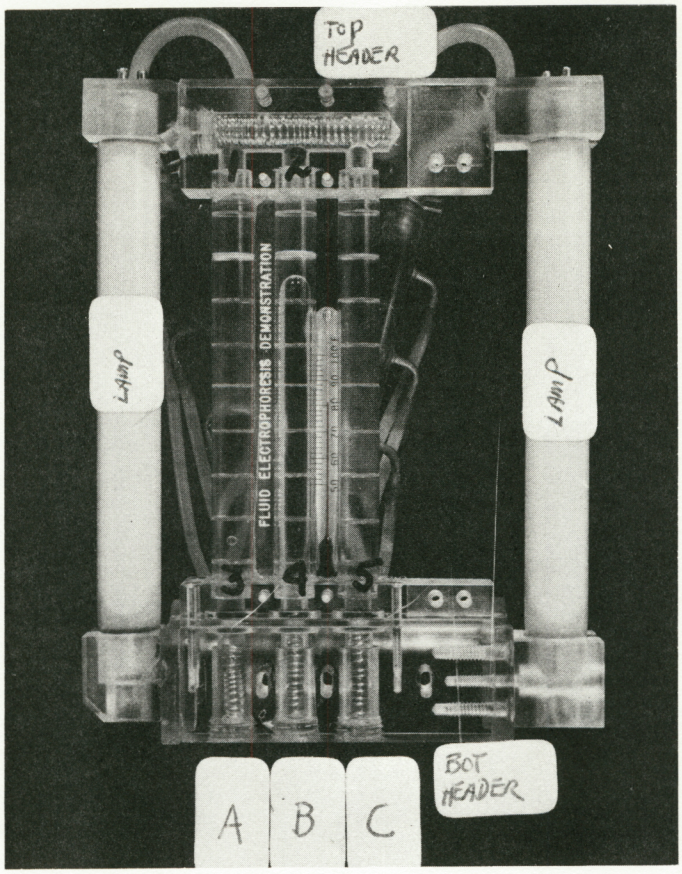
Cracks occurred on CCl_4 test after surface was cut. Crack orientation indicated longitudinal stress under outer surface layer which showed a shallow circumferential stress. These tubes should be stress-reieved by annealing as in P II.1.5.

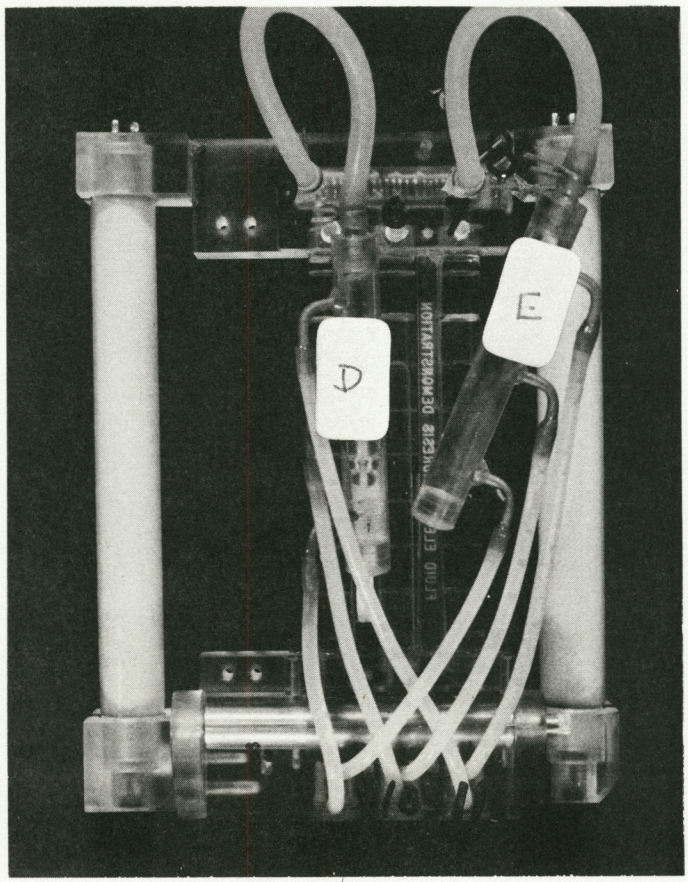
1.7 Joints Bonded with Epoxy-Versamild # 140 adhesive were essentially stress free and did not contribute to cracking.

III Compatibility of the Borate Buffer Solution Used In This Cell With Lexan

1.0 Cricitcal strain tests at 120°F show that this buffer solution does not produce eracking at any stress level below 5000PSI

and should not have been a contributor to any cracking with the possible exception of that which occurred at the notch concentrated stress associated with cracks # 1 and # 2 (P I.1.1).


TOP HEADER
LAMP
LAMP
FLUID ELECTROPHORESIS DEMONSTRATION
3
4
5
BOT HEADER
A
B
C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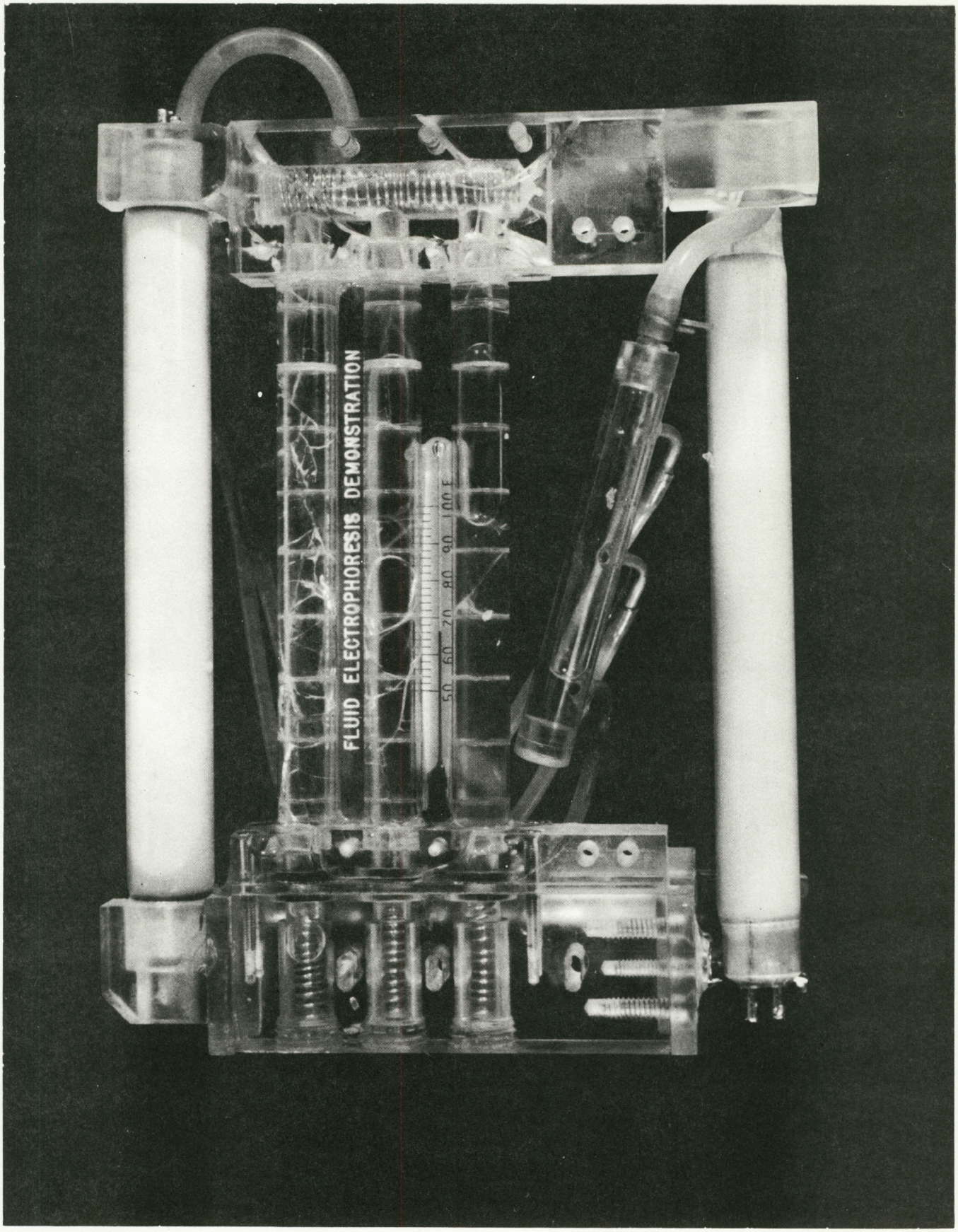
FLUID ELECTROPHORESIS DEMONSTRATION